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TECHNICAL BULLETIN No. 6

SEPTEMBER, 1947

VITAMIN VALUES OF FOODS IN HAWAII

CAREY D. MILLER
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KISAKO YANAZAWA

UNIVERSITY OF HAWAII AGRICULTURAL EXPERIMENT STATION

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PREFACE

Many of the data on the vitamin content of foods grown, used, and/or produced in Hawaii which are given in this bulletin were first published in mimeographed form in April, 1942, by the Hawaii Agricultural Experiment Station, as Progress Notes No. 36 to make available information on local and semitropical foods that might be of use in connection with food planning during the war.

Because of the many requests for these Notes, a revision with some additional data was published in June, 1944.

This bulletin includes all data contained in Progress Notes No. 36, material that could not be included at that time, and material which has accumulated since.

Eva Hartzler, Associate Nutritionist, and Winifred Ross, Junior Nutritionist, have carried out experiments which provide a portion of the data included in this bulletin which did not appear in the first edition of Progress Notes No. 36. The authors are indebted to Miss Hartzler and Miss Ross for critical reading of the manuscript.

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August, 1946

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INTRODUCTION

IN ADDITION to many familiar American foods, there are available in Hawaii many foods of tropical and semitropical origin, and foods characteristic of the diets of the racial groups which make up the population of the Islands. To provide information about the vitamin content of these foods, which are not included in published tables but which are of importance in local diets, and to determine the comparative vitamin value of fruits and vegetables produced locally with those produced elsewhere, a general survey of the vitamin values of foods in Hawaii was undertaken. The results of this survey are presented in this bulletin.

Following a brief discussion of the vitamins and their importance in nutrition, and a statement of the methods used, the vitamin A, vitamin B₁ (thiamine), and vitamin C (ascorbic acid) contents of a large number of foods are summarized in table form, with a brief discussion of the results. Results are also given for some detailed ascorbic acid studies and for a few foods tested for vitamin D. Detailed supporting data are included in the appendix.

BRIEF SURVEY OF PRESENT KNOWLEDGE OF VITAMINS

VITAMINS DEFINED

Vitamins are nutritionally essential substances, required in relatively small amounts, that are neither minerals, carbohydrates, lipides, proteins, nor their derivatives (47). Chemically, each vitamin has a different structure unrelated to that of others. The system of designating the vitamins by letters of the alphabet or as specific factors, initiated shortly after their discovery, has continued until each vitamin has been chemically identified. For vitamins A and D, no generally accepted chemical names have ever been adopted though their chemical structures have been known for 10 years or longer.

CLASSIFICATION AND NOMENCLATURE

The vitamins are commonly classified in two general groups—water-soluble and fat-soluble. The most recent reviews (7, 12, 38, 47) include 11 chemically identified substances as the water-soluble vitamins and four as fat-soluble vitamins, with as yet unidentified substances in each group.

The water-soluble vitamins are further divided into "B and C families." Under the B vitamins are listed thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folic acid, *p*-aminobenzoic acid, choline, and inositol. The close association of the first eight in biological tissues has been demonstrated and an even closer relationship of the first four (47). Williams has pointed out the universal distribution of the B vitamins in all living matter from the lowest organisms up the scale to the most highly developed forms and the ability of the B vitamins to stimu-

late growth in all forms of life. He states that "presumably they are constituents of fundamental catalytic systems which are essential to living processes" (47).

Ascorbic acid and vitamin P constitute another group of water-soluble vitamins. Vitamin P, though not fully identified, is conceded by many to be a substance (or substances) that affects capillary resistance (18, 38). If sufficient fruits and vegetables are consumed to supply adequate amounts of ascorbic acid, there should also be sufficient vitamin P, because it occurs naturally in many of these foods.

The fat-soluble vitamins are A, D, E, and K, only one of which, vitamin E, is commonly referred to by its chemical name tocopherol. One deterrent to names other than the alphabetical ones for the fat-soluble vitamins is their occurrence in several different forms. Thus far it has been clearly established that there are two naturally occurring and effective forms of vitamin A, two of vitamin D, three of vitamin E, and three of vitamin K, which are referred to as A₁, A₂, D₂, D₃, etc. (7, 42). In addition, there are other substances, natural and synthetic, that exhibit some of the properties of the well-established forms. For example, in 1939, Bills (2) stated that at least 10 compounds had been shown to have antirachitic properties, yet today (1946) the two most important forms are referred to as D₂ and D₃.

REASONS FOR STUDYING ONLY FOUR VITAMINS

The occurrence of four vitamins—vitamin A, thiamine, ascorbic acid, and, to a much smaller extent, vitamin D—has been studied in foods produced or widely used in Hawaii. These vitamins are among the oldest historically, and satisfactory methods for the determination of only these four had been established when the studies reported here were begun in 1937.

NUTRITIONAL SIGNIFICANCE OF THE VITAMINS

The physiological functions of, the occurrence and stability of, and the requirements and allowances for each of these four vitamins will be briefly discussed. For additional information on these and other vitamins, the reader is referred to the most recent reviews, especially the latest numbers of the *Annual Review of Biochemistry*, and *Vitamins and Hormones*.

VITAMIN A

Vitamin A promotes growth and helps to keep the epithelial tissues of the skin and of the mucous membranes lining different parts of the body in a healthy condition. It is necessary for the formation and the maintenance of good teeth. It is also essential for normal vision, and a deficiency will result in night blindness.

If more vitamin A is taken each day than is needed, the body is able to store it.

Vitamin A is a practically colorless crystalline substance soluble in fats and fat solvents and is found only in foods of animal origin. The vitamin A values of plants are due to their content of orange-yellow pigments, or

carotinoids, which can be changed to vitamin A in the animal body. The term Vitamin A is used to designate both the true vitamin A and the orange-yellow pigments that can be changed to vitamin A. Just how well the carotinoids are utilized by human beings on a normal diet has not been fully determined, but it seems certain that man does not use them so efficiently as rats, depleted of vitamin A stores, which are used for the biological determination of vitamin A values of foods. Recent reports indicate that 35 to 50 percent of the carotene of vegetables may be absorbed (12) and that only 15 to 20 percent of that absorbed may be converted to vitamin A (7). Such experiments indicate the desirability of eventually reporting the carotene and vitamin A values of foods separately.

Green and yellow vegetables are, nevertheless, excellent and economical sources of the precursor of vitamin A. White or bleached vegetables contain little or none of the precursor. Deep green vegetables, such as watercress, Chinese spinach, and luau, contain 50 to 200 times as much provitamin A as faintly green or bleached vegetables, such as cabbage or lettuce.

In either form, vitamin A in foods is relatively stable and is not readily destroyed by the usual methods of food preservation and preparation. Vitamin A *per se* is readily destroyed by oxidation, however, and in rancid fats or foods containing rancid fats, the chemical constitution of vitamin A is readily altered and its physiological effectiveness is lost.

THIAMINE (VITAMIN B₁)

Though each of the B vitamins is known to have special physiological functions, thiamine has many properties in common with some of the others and some functions are believed to be interrelated. Thiamine is essential for growth. Its role, as well as that of the other B vitamins, in the complex metabolism of every cell has not yet been fully elucidated, though thiamine is known to be involved in an enzyme system essential for the metabolism of carbohydrates. Along with other B vitamins, it affects appetite and helps to keep healthy the digestive tract, the skin, the eyes, and the nervous system.

The chemical synthesis of thiamine by Williams in 1936 stimulated further research regarding its function and occurrence, with the result that chemical methods for determining this vitamin are replacing the various biological methods. Because thiamine in foods usually occurs in extremely small amounts—0.1 to 4 parts per million (46)—problems related to its extraction from foods prior to chemical determination have proved to be very troublesome.

Since thiamine is stored only to a limited extent and the excess over daily needs is normally eliminated in the urine, a proper choice of foods to insure a regular intake is recommended.

The best food sources of thiamine are the whole grains, lean pork, legumes, and nuts. As a class of foods, fresh vegetables are not considered a good source of thiamine. However, when compared on the calorie basis, such foods as potatoes, sweetpotatoes, taro, poi, pumpkin, and winter-type squash are remarkably good sources. (Compared on the uncooked basis, 100 calories of brown rice will furnish 81 micrograms of thiamine

and 100 calories of white potatoes will furnish 130 micrograms.¹) Such products as wheat germ, rice middlings, and certain types of dried yeast constitute the most concentrated naturally occurring sources of thiamine and are also good sources of some of the other B vitamins. Thiamine is sensitive to heat, alkali, and oxidation. The greatest losses of thiamine occur as a result of (1) milling grains if all the bran and germ are discarded, (2) heating to a high temperature, such as is required in canning and cooking some foods, and (3) discarding water in which foods have been soaked, cooked, or canned.

ASCORBIC ACID (VITAMIN C)

The first vitamin chemically identified and synthesized was ascorbic acid. It is a white crystalline material readily soluble in water and is more readily destroyed by oxidation than any of the other vitamins. Light, heat, and alkali increase the rate of destruction by oxidation. Ascorbic acid is more stable in acid than in neutral media.

The exact role of ascorbic acid in the oxidation-reduction processes in the animal body has not yet been fully determined (18, 38, 47), but it is generally conceded to be an important one. It has been clearly established, however, that ascorbic acid controls the production and normal functioning of material around and between the body cells (intercellular substance). When this function fails in the young or old, scurvy will occur. Man shares with the monkey and the guinea pig the inability to make his own ascorbic acid. It has been proved that other animals, such as the dog, rat, and cow, have a definite need for ascorbic acid but that they are able to synthesize it and store small amounts in their livers. Because the human body can store little ascorbic acid, foods that will supply an adequate amount should be included in the diet each day.

Although ascorbic acid is necessary for growth and health at all ages, it is especially important for the bottle-fed baby, because sterilized or pasteurized milk mixtures contain little or no ascorbic acid, whereas human milk supplies adequate amounts for the nursing infant if the mother has a proper diet.

Dry grains and cereal products, legumes, meats, fish, eggs, and all fats furnish no ascorbic acid. Fruits and vegetables, the chief food sources, vary greatly in their content of this vitamin. In addition, methods of storage, transportation, and preparation all influence markedly the ascorbic acid content of fruits and vegetables as they reach the consumer's plate.

VITAMIN D

The exact manner in which vitamin D functions to promote normal calcification of teeth and bones is still in dispute (7, 42), but that it prevents rickets in infants and growing children is clearly established. It is

¹ These calculations are based on data given in Miscellaneous Publication 572. If the figures for thiamine for these two foods in the cooked state which are given in table 1, page 16, of this bulletin are used, the results are even more favorable for potatoes, as 100 calories of cooked brown rice furnish 73 micrograms of thiamine, and 100 calories of cooked potatoes 163 micrograms.

believed that all mammals and all birds require vitamin D for normal bone growth.

According to Jeans, not only infants but all growing children and youths need vitamin D for normal calcification of bones (17). Some scientists believe normal adults have a definite need for vitamin D, although the requirements are difficult to determine (41). Even occasional exposure of the skin to sunshine will apparently provide adequate vitamin D for the normal adult. The National Research Council has proposed allowances for infants, children, and youths to 20 years, and for pregnant and lactating women (36).

Like vitamin A, vitamin D has known precursors. Irradiation with ultraviolet light will change ergosterol, a sterol occurring in plant tissues, to vitamin D₂, and 7-dehydrocholesterol, which occurs in the skin of animals, to vitamin D₃. Both effectively prevent rickets in infants and children. Both the precursors and the vitamins have been chemically identified and their structures established.

Like other fat-soluble vitamins, the D vitamins can be stored in the body in excess of daily needs. They are more stable than vitamin A but are readily destroyed in rancid fats or food mixtures containing rancid fat.

Fruits, vegetables, cereals, meats, and vegetable fats are normally devoid of vitamin D. For this reason we have tested only a few foods for their vitamin D content. The Food and Drug Administration of the federal government requires proper labeling of all cereals, vegetable fats, and milk given antirachitic properties by exposure to ultraviolet light or by the addition of a concentrated source of vitamin D.

Chemical methods for the determination of vitamin D are still unsatisfactory, and biological methods with rats or chicks are commonly used.

REQUIREMENTS AND ALLOWANCES

Daily allowances for six of the well-established and more fully studied vitamins have been recommended by the Food and Nutrition Board of the National Research Council (36). These allowances are greater than the supposed requirements for several reasons. In addition to the natural variations in the vitamin content of foods, due to such factors as variety and environment, there may be large losses as a result of storage, cooking, and processing, which affect the vitamin content of the foods as eaten. Moreover, absorption and utilization may differ even in normal individuals. Differences in metabolic rates, febrile conditions, and infections may further change the requirements. Experiments with humans and other animals have clearly demonstrated an increased need for vitamins during pregnancy and lactation.

The daily allowances recommended by the National Research Council for the standard man for three of the vitamins discussed in this bulletin are 5,000 International Units of vitamin A, 1.5 milligrams of thiamine, and 75 milligrams of ascorbic acid. (No allowance for vitamin D for the standard man has been established.) For complete data on the allowances for these and other vitamins, for different ages and sexes, the tables prepared by the Council should be consulted (36).

STUDY OF THE VITAMIN VALUES OF FOODS IN HAWAII

METHODS

FOOD SAMPLES USED FOR VITAMIN ASSAYS

The edible portions of foods, prepared as for human consumption, were used for all feeding experiments. As the biological assay periods for vitamin A and for thiamine were of 3 weeks' duration and all animals could not be started on supplements at the same time, it was often necessary to obtain foods over a period of 4 or 5 weeks. Therefore, most of the vegetables were obtained from the markets and the varieties were frequently unknown. In some instances, different varieties that had much the same appearance may have been used for one feeding test. If the vegetables were obtained from the Station farms or direct from the grower and the varieties were known, they are given either in table 1 or in the Appendix, pages 44 to 48. If no specific descriptions are given for the foods, it should be assumed that they were of good market quality, representative of the type of food listed.

Unless otherwise indicated, all vegetable samples for vitamin A and thiamine assays were fed in the cooked state and all fruits in the raw state.

Foods for the ascorbic acid assays are described in Appendix II unless only one sample of good market quality food was used.

Preparation of vegetables for vitamin assays

Edible portions of the vegetables were washed and freed of excess water by shaking or patting between clean towels. Unless otherwise indicated they were then cooked as follows: the vegetables were put into aluminum pans, 3 inches deep and 9 inches in diameter, which were placed on a wire rack 1 inch above the bottom of an 11-quart pressure cooker containing hot water to a depth of one-half inch. Sometimes a single pan was used; if two pans were used, one was placed above the other. The lid was placed on top but was not screwed in place. *The vegetables were not cooked under pressure at any time.* The cooking period was reckoned from the time steam began to issue from the pet cock. This procedure afforded a uniform method that could be readily duplicated and prevented loss of juices and any marked changes in weight. Fresh vegetables were cooked twice each week, on Mondays and Thursdays. After being cooked, they were chopped or finely cut to make a uniform sample and stored in the refrigerator in glass jars with tight-fitting covers. Each sample was normally used 3 days for feeding.

The following vegetables were cooked by steam (no pressure) in a pressure cooker:

For 5 minutes: bean sprouts (for ascorbic acid), green onions, watercress.

For 10 minutes: bean sprouts (for vitamin A and thiamine), beet greens, bitter melon green leafy tips, cabbage (all kinds), chard, cowpeas green leafy tips, horseradish tree pods, hyacinth beans, jute, purslane, Chi-

nese peas (edible pod), Chinese spinach (amaranth), squash tips, swamp cabbage, turnip greens, yam bean root.

For 15 minutes: green beans (two varieties), Goa beans, broccoli, cowpeas, green peppers, horseradish tree leaflets and tender tips, Malabar nightshade, okra (for ascorbic acid), pigeonpeas, summer squash (pattypan and zucchini), sweetpotato tops.

For 20 minutes: asparagus, cooking banana (in skins), yellow wax beans, bitter melon fruit (25 minutes if old), carrots, celery, daikon, eggplant, okra, winter-type squash and pumpkin, treefern fronds, turnip greens.

For 30 minutes: lima beans (40 minutes if more mature), chayote (unpeeled), Chinese preserving melon, lotus root.

For 60 minutes: taro leaves (luau).

Some vegetables were cooked by other methods, as follows:

Beets in skins with tops and tails were covered with boiling water and cooked for 40 to 45 minutes. Beets were stored in refrigerator and peeled daily as needed.

Belembe (Tahitian taro) was cooked in a small amount of water in a covered saucepan for 10 to 15 minutes, until all water was absorbed or evaporated.

Breadfruit was baked in the skin for 1 hour at a temperature of 350° F.

Corn on the cob, with silk and husks removed, was cooked in boiling water for 5 minutes.

Soybeans (mature dry) were cooked without previous soaking for 1 hour at 15 pounds pressure in a pressure cooker.

Soybeans (fresh green) were cooked in the pods in boiling water to cover for 25 to 30 minutes (some varieties required longer cooking than others).

Japanese taro (unpared) was covered with boiling water and cooked for 30 to 35 minutes.

Potatoes (unpared) were covered with boiling water and cooked for 20 to 30 minutes, depending on the size.

Sweetpotatoes (unpared) were covered with boiling water and cooked for 30 to 50 minutes, according to size and variety.

Preparation and sampling of other foods

Eggs were immersed in tap water in a small covered saucepan. The water was brought to the boiling point, the pan removed from the heat, and the water allowed to become almost cool before removing the eggs. The yolk was mashed with a silver fork and thoroughly mixed with the finely chopped white.

Macadamia nuts (commercially prepared). The dried kernels were cooked in refined coconut oil at 275° F. for 12 to 15 minutes.

Peanuts (Spanish variety), commercially prepared, were roasted in a hydrogenated vegetable fat for 10 minutes at 310° F.

Pork shoulders were obtained from pigs that had been used for fattening experiments in the Experiment Station Animal Husbandry Department. The figures for raw pork were obtained by one bio-assay of a ground, thoroughly mixed sample prepared from the lean meat (freed of visible fat) from two shoulders, each shoulder from a different pig. The values for cooked pork shoulder, both grain- and garbage-fed, represent the averages of two bio-assays. The pork used for the first assays of the grain- and garbage-fed pork was a mixture of the lean meat from two shoulders in each case, each shoulder from a different pig. The second bio-assays on grain- and on garbage-fed pork were each made on a single shoulder. The shoulders, weighing 6.5 to 9.5 pounds, were roasted for about 3 hours at an oven temperature of 350° F.

Imported brown rice of the short-grain variety (labeled Fancy California Brown Rice) was cooked in the oriental manner. A relatively small amount of tap water was added to the rice in a tightly covered saucepan; the water was brought to a boil and boiled gently until most of the water was absorbed or had evaporated; the heat was then reduced so that the rice steamed for 30 minutes (total cooking time about 50 minutes). The figures for raw rice were calculated from the bio-assays using cooked rice.

The partially polished rice, made locally from imported California brown rice, retained a portion of the pericarp and of the embryo. It was cooked in a manner similar to the brown rice, but the total cooking time was about 40 minutes.

White polished rice, also imported, was cooked in the oriental manner for a period of about 30 minutes.

The so-called black rice, imported from the Philippines, was cooked in the same manner as the brown rice.

BIOLOGICAL DETERMINATION OF VITAMINS

All experimental rats were raised in the Station laboratory. When used for testing the vitamin content of foods, the rats were kept in individual cages with raised screen bottoms and were allowed tap water and the basal diets *ad libitum*. Wherever it is stated that supplements were fed daily, the word "daily" is to be understood to mean every day except Sunday.

Vitamin A

Vitamin A values of foods were determined by bio-assay. Rats, depleted of vitamin A stores, were fed food supplements and their growth response was recorded and compared with a curve of reference for rats of the Station colony. Details of the procedures, diets, weight gains, and results are given in Appendixes I and III.

United States Pharmacopeia reference cod liver oil No. 2 was used to establish the curve of reference and for the positive controls from 1938 to 1944. Although fresh samples were obtained at suitable intervals and the oil was not used beyond the dates recommended, by 1943 it became apparent that the oil was less potent than when first used. In 1945 vitamin

A acetate² supplemented with tocopherol was used as a standard for a small series of rats. Data on the growth curve of these rats are given in table E, page 51. Details of the diet and procedure are given in Appendix I, page 42.

Unless otherwise indicated, all vegetables were fed in the cooked state, whereas with few exceptions fruits were fed in the raw state. The amounts of the food supplements were usually so small that they were weighed on a chainomatic balance and fed two or three times a week.

Positive controls were fed the oil solutions of reference cod liver oil or of vitamin A acetate, measured by means of an "Agla" micrometer syringe apparatus, two or three times a week.

Thiamine

The growth response of groups of rats depleted of thiamine stores and then fed various food supplements was compared with curves of response established in this laboratory for rats fed pure thiamine chloride after a first and after a second depletion of thiamine stores. These methods have been fully described elsewhere (22, 23).

The weights of the food supplements, the number of rats used, and the mean gains of groups of rats are given in Appendix IV, page 52. Negative controls fed the basal diet only and positive controls fed thiamine were used for each series. Similar detailed data have been previously published for a number of the foods listed in table 1 (21, 24, 26, 27, 29, 30, 31) and to save space are not repeated here.

Unless otherwise indicated, all vegetables were fed in the cooked state and fruits in the raw state. Thiamine, in a 10 percent alcoholic solution, was administered directly into the mouths of the rats by micropipettes.

Ascorbic acid

Because the dye titration method indicated such low ascorbic acid values for Chinese and Bluefield bananas, beets, and beet greens, these four foods were tested on guinea pigs, using the Höjer method of histological examination of the lower incisor teeth (14).

As the results of the feeding experiments confirmed the values determined by chemical means, the details are not included here. Biological tests on a number of Hawaiian-grown fruits have previously been reported elsewhere (28).

Vitamin D

Rats were fed Steenbock's rachitogenic diet No. 2965 for 21 days. Twenty-one-day and 31-day negative controls were taken from each litter. Supplements were fed for 8 days, then only the basal diet for 2 days. The rats were killed and the tibias removed for the line tests, which were graded according to the method of Bills *et al.* (3).

² The vitamin A acetate was purchased from Distillation Products Inc., Rochester, New York.

A curve of response established for rats of this laboratory by using about 130 animals fed the United States Pharmacopeia reference cod liver oil as a source of vitamin D has been published elsewhere (26). The results in table 2, page 23, were obtained by using this curve and the ratings based on the degrees of healing for groups of rachitic rats fed the various supplements.

CHEMICAL DETERMINATION OF VITAMINS

Ascorbic acid

Some form of the dye (2, 6—dichlorophenolindophenol) titration method was used for the determination of ascorbic acid values of all foods except those that yielded colored extracts, for which the photoelectric colorimeter was used.

At first, samples were ground in glass mortars with acid-washed sand, using a mixture of 2 percent HPO_3 and 8 percent HAc according to procedures recommended by Bessey and King (1), and Musulin and King (35). Later 3 percent HPO_3 only was used and the extracts were prepared by using a Waring blender and filtering. Still later, 0.5 percent oxalic acid was used as the extractant (39). The photometric method used was the procedure recommended by Morell (33).

Foods that are commonly eaten in the cooked state or which may be eaten in the raw or cooked state were assayed before and after being cooked.

For description and preparation of foods used for the ascorbic acid assays, see Appendix II, pages 44 to 48.

RESULTS AND DISCUSSION

Vitamin values for foods produced (or widely used) in Hawaii given in table 1 include vitamin A values for 85 foods; thiamine, 90 foods; and ascorbic acid, 110 foods. If values for similar foods appear in Miscellaneous Publication 572 of the Bureau of Human Nutrition and Home Economics, U.S.D.A. (5), they have been compared with the Hawaiian values.

VITAMIN A

The values for 23 foods appearing in table 1 were compared with the average figures in Miscellaneous Publication 572. All values for the Hawaiian foods were greater except for oranges, pineapple, and potatoes. The value for carrots was the same as the published value. Hawaiian-grown oranges are much lighter in color than California oranges and therefore might be expected to have a lower vitamin A value.

The reason for the generally higher vitamin A values may be the result of feeding cooked instead of raw products. Vegetables and fruits relatively high in vitamin A values are usually fed to the experimental animals in such small quantities that it is necessary to chop the foods very fine and mix them well to get a uniform sample. Such a method of preparation results in a thorough exposure to air and if the enzymes have not been destroyed, oxidation probably takes place readily.

The results of supplying food in the raw and cooked states may be illustrated with guavas, which require considerable manipulation to prepare a uniform sample for feeding because it is necessary to free the pulp from the seeds.

A fresh lot of guavas was obtained once each week and kept in the refrigerator until used. The products for feeding were prepared twice a week. For the fresh product, the pulp containing the seeds was removed from one half of each guava, and was put through a fine strainer to remove the seeds. The guava rinds were chopped fine, then mashed and mixed with the strained pulp, and stored in a covered glass container in the refrigerator until used.

For the cooked product, the other half of each guava was sliced, and after the addition of a measured amount of water, the guavas were cooked for 10 minutes. They were then put through a fine strainer, such as was used for the fresh guavas, and the product stored in a like manner. Weights taken before and after cooking made it possible to calculate the equivalent fresh weights. The raw and cooked products were fed in the usual manner. Taking into account the weight of edible guava and seeds, we calculated the results to be as follows:

	I.U. Vitamin A/100 grams
Raw whole guavas	230
Cooked whole guavas	570
Raw guava pulp	260
Cooked guava pulp	640

It is doubtful that the cooking process would have so greatly increased the absorption of the provitamin, so it seems reasonable to assume that the oxidation which took place in the presence of the enzymes of the fresh guavas accounted for the destruction of the carotene.

The vitamin A values of all foods tested followed the usual pattern—thin green leaves and deep yellow foods showing the highest values. Fruits and vegetables that were light yellow or pale green proved to have less vitamin A, and white fruits and vegetables had none.

The contrast between the pale green or bleached Chinese cabbage and head cabbage, and the green leafy types is striking. The figures in table 1 indicate that the latter have more than 100 times the vitamin A value of the two bleached types.

Some foods contained too little vitamin A to demonstrate by the feeding technique used; that is, the rats fed the supplements failed to gain weight and died as soon as the controls. Foods tested for vitamin A with negative results were carissa, coconut, lotus root, macadamia nuts, miso, peanuts, and tamarind.

Foods that showed no evidence of color, either green or yellow, such as daikon, litchi, and soursop, were not tested but were assumed to contain no vitamin A. The vitamin A values for both these foods and those tested with negative results are indicated by 0 in table 1.

TABLE 1. Vitamin A, thiamine, and ascorbic acid values of foods in Hawaii (per 100 grams of edible food).

FOOD	VITAMIN A*	THIAMINE* (VITAMIN B ₁)	ASCORBIC ACID (VITAMIN C)	
			Raw	Cooked
	<i>I. U.</i>	<i>Micrograms</i>	<i>Milligrams</i>	<i>Milligrams</i>
Asparagus, green	3,500	145	29	20
Avocado (winter and summer varieties)	150-400	80-150	5-15	---
Banana bud	---	---	1	1
Banana†	---	---	---	---
Baking	900	40	16	16
Bluefield	600	‡	6	---
Bean sprouts, mung	140	100	15	6
Beans, green	---	---	---	---
Kentucky Wonder	2,300	100	13	11
Lualualei	1,500	135	12	10
Beans, green lima	500	234	36	28
Beans, soy (see Soybeans)	---	---	---	---
Beans, yellow wax	260	75	20	11
Beet greens	8,800	48	9	2
Beets	50	30	6	3
Belembe (Tahitian taro)	20,000	85	96	38
Bitter melon	---	---	---	---
Fruit	160	40	63	33
Leafy tender tips	20,000	112	88	20
Blackberries, evergreen	---	---	10	---
Breadfruit	---	---	---	---
Ripe, cooked	100	115	26	16-20
Mature green	---	---	50	---
Broccoli	7,250	87	102	83
Burdock root (gobo)	---	---	‡	‡
Cabbage	---	---	---	---
Chinese (celery cabbage)	40	---	37	25
Green mustard	11,000	55	75	48
Head	90	33	52	35
White mustard	9,000	34	47	40
Raw, pickled with salt	---	30	---	---
Raw, pickled with salt and rice bran	---	260	---	---
Cactus fruit	---	---	28	---
Carambola	900	35	35	---
Carissa	0	---	54	---
Carrots	12,000	45	6	5
Celery	---	---	12	8
Chard	6,500	58	15	5
Chayote	400	---	9	---
Coconut	---	---	---	---
Water	0	---	2	---
White meat, fresh mature	0	110	0	---
White meat, immature	---	---	3	---
Corn, sweet, U.S.D.A. No. 34 (faintly yellow)	120	---	---	---
Cowpeas	---	---	---	---
Fresh pods	4,000	150	25	16
Fresh seeds	800	400	20	15
Tender tips	---	---	36	25
Cucumber	---	---	---	---
Pared	---	---	7	---
Unpared	---	---	10	---

* Vitamin values for vitamin A and thiamine were determined on vegetables in the cooked state and fruits in the raw state unless otherwise indicated.

** --- indicates that no determinations have been made. In some instances the nature of the food made it doubtful that any vitamin could be demonstrated biologically, e.g., vitamin A of rice. In other cases the food is normally not used in the cooked state, e.g., avocado.

† For ascorbic acid content of other varieties, see table 3, p. 25.

‡ Too little to determine by the methods used.

TABLE 1 (continued)

FOOD	VITAMIN A	THIAMINE (VITAMIN B ₁)	ASCORBIC ACID (VITAMIN C)	
			Raw	Cooked
	<i>I. U.</i>	<i>Micrograms</i>	<i>Milligrams</i>	<i>Milligrams</i>
Daikon (Japanese radish)				
Bran-salt pickled, raw				
Commercial imported				
(Takuan)		135		
Homemade		500		
Raw	0	20-40	27	
Eggplant				
Long				
Bran-salt pickled, raw		130		
Raw, with skin		70	3	2
Round	200	90	1	< 1
Egg yolk	5,000			
Figs, Brown Turkey	80	30	2	
Fruit juices and nectars,				
commercial*				
Goa or winged bean	700	171	< 1	0
Gourd, white flowered			9	6
Grapes, Isabella				
Pulp only			2	
Whole			5	
Guava, Cattley				
Red (Strawberry)			33	
Yellow			53	
Guava, common				
Juice and pulp, home-canned				
and bottled, 50 samples			30-130	
Seeds removed	600	40		
Whole			70-350	
Horseradish tree				
Leaflets and tender tips	35,000	227	134	36
Pods	650	54	172	126
Hyacinth beans	560	118	13	7
Java plum (jambolan)				
Purple flesh			27	
White flesh			31	
Jute (Filipino spinach)	6,000	73	36	1
Ketambilla (Ceylon gooseberry)			82	
Kiawe bean meal	60			
Lettuce, Manoa (semi-head),				
raw	6,600		8	
Lime juice, 4 varieties**			10-30	
Litchi, Kwai Mi	0		64	
Longan			72	
Lotus root	0	70	42	36
Macadamia nuts				
Raw	0	486		
Roasted	0	408		
Malabar nightshade	13,000	115	166	86
Mango				
Different varieties				
Green or half ripe†			40-180	
Ripe‡			5-140	
Pirie	6,000	60	15	
Melon, Chinese preserving			18	16
Milk				
Pasteurized				< 1
Raw	300	28	1	

* For ascorbic acid values, see table 7, p. 33.

** See table 3, p. 25.

† See table 4, p. 26.

‡ See table 3, p. 25.

TABLE 1 (continued)

FOOD	VITAMIN A	THIAMINE (VITAMIN B ₁)	ASCORBIC ACID (VITAMIN C)	
			Raw	Cooked
	<i>I. U.</i>	<i>Micrograms</i>	<i>Milligrams</i>	<i>Milligrams</i>
Miso (see Soybean products)				
Mountain apple	Trace	15	20	
Mulberry, black			12	
Ohelo berries			6	
Okra	1,000	90	13	9
Onions				
Dry	0		7	
Green	8,200	60	25	20
Opihi (Hawaiian limpet), raw	4,000	0	0	
Orange juice, Hawaiian	70	50	65	
Paiai (see Taro, Hawaiian)				
Papaya, Solo and large varieties	4,000	30		
40 samples, Kailua			59-118	
Average			88	
45 samples, Poamoho			60-122	
Average			81	
Passion fruit, fresh	550		20	
Peanut butter, average of four commercial brands		378		
Peanuts				
Raw	0	580		
Roasted in oil	0	166		
Peas, Chinese (edible pod)		160	60	47
Pepper, green bell	1,300		90	88
Pigeonpea, green shelled	1,700	486	33	49
Pineapple, Cayenne, fresh pulp	110	75	8	
Pineapple bran, dried	5,000			
Plum, red, Methley	500		2	
Poha	4,000	150	42	
Poi (see Taro, Hawaiian)				
Pomelo			68	
Pork shoulder*				
Grain-fed				
Cooked		500		
Raw		865		
Garbage-fed, cooked		340		
Potato				
Bliss Triumph	Trace	138		
3 days after harvesting			34	32
Stored 2 months**			15	13
New, imported			13	
Pumpkin (see Squash)				
Purslane	3,500	42	20	12
Rice				
Black, dry weight basis		240		
Brown, California				
Cooked weight basis		100		
Dry weight basis		260		
Partially polished, dry weight basis		200		
Middlings		3,600		
White, California, washed and cooked		†		
Roselle			10	
Sesbania flowers, white			73	37
Soursop	0	45	20	
Soybean products				
Aburage (fried curd)		32		

* Freed of visible fat.

** See table 9, p. 35.

† Too little to determine by the methods used.

TABLE 1 (continued)

FOOD	VITAMIN A	THIAMINE (VITAMIN B ₁)	ASCORBIC ACID (VITAMIN C)	
			Raw	Cooked
	I. U.	Micrograms	Milligrams	Milligrams
Soybean products (cont.)				
Kirazu (curd residue)		155		
Miso (fermented rice and soybeans)		133		
Tofu (curd)	0	54		
Tonyu ("milk")		75		
Soybeans				
Dry mature, raw, soaked		359‡		
Green	1,800	190	33	20
		raw 310		
Spinach, Chinese (amaranth)	13,900	35	22	9
Squash				
Summer				
Pattypan			18	
Zucchini	700	54	15	2
Winter-type or pumpkin*				
Flowers	1,700	0	28	5
Fruit	12,000	140	15	14
Leafy tender tips	2,700	162	11	1
Strawberry				
Red (cultivated)			66	
White (small, wild)			33	
Surinam cherries	2,000		20	
Swamp cabbage	10,600	80	44	10
Sweetpotatoes				
Deep yellow	12,000			
Light yellow	2,400	112	6	4
Tops	8,500	176	11	2
Tamarind				
Green			1	
Ripe	0	300	0	
Tangelo			33	
Taro, Hawaiian				
Leaves (luau)	20,000	150	52	43
Paiai (cooked taro) 30 percent solids	65	90		9
Poi, 17 percent solids	35	48		5
Steamed corm	75	105	5	5
Taro, Japanese (dasheen)	80	125	4	4
Tofu (see Soybean products)				
Tomatoes				
Globe				
Imported, average 12 samples			14	
Local, 72 samples purchased from markets			10-25	
Average			15	
Pear-shaped, raw	1,200	140	26	
Tree fern fronds				
Large (<i>Cibotium chamissoi</i> Kaulf.)			1	2
Small (<i>Sadleria</i> sp.)			5	10
Turnip greens	11,000		67	40
Vi			42	
Watercress	5,000**	75	56	51
Watermelon			6	
Yam bean root			3	2
Yeast, dried (H.S.P.A. Expt. Sta.)		5,280		

* The terms pumpkin and squash are often used interchangeably. It was not possible to have our samples identified botanically as stems, leaves, and flowers were not always available.

** Raw.

‡ Equivalent to 835 micrograms/100 grams, dry weight.

THIAMINE

The thiamine values for only 23 of the foods appearing in table 1 were compared with average values given in Miscellaneous Publication 572. Nine foods with lower than average values were asparagus, bananas, cabbage, carrots, celery, milk, okra, orange juice, and brown rice. Six foods with higher than average values were green beans, peanut butter, potatoes, squash or pumpkin, sweetpotatoes, and tomatoes. Eight foods with values considered to be the same (within 5 micrograms per 100 grams) were avocado, lima beans, beets, beet greens, broccoli, chard, eggplant, and pineapple.

Considering the different varieties of fruits and vegetables tested and the different assay techniques probably used to obtain the average values, our results and the published figures agree remarkably well. For only about seven of the foods were the values strikingly different. We were unable to demonstrate any thiamine in the Bluefield variety (also called Gros Michel) of bananas. Although the rats ate the fresh fruit readily, a daily supplement of 6 grams caused no increase in weight. The published averages for head cabbage and carrots are almost twice those determined for the Hawaiian-grown vegetables.

Our value (average of two tests) for the thiamine of mixed herd milk (raw) produced on the University Farm is much less than the reported average figure. This suggests the advisability of making a detailed chemical study of milk in Hawaii to determine (1) whether all milk produced in Hawaii shows similar values and (2) if possible, whether climatic or other environmental factors influence the thiamine content.

Tests on Hawaii orange juice, prepared fresh daily, were made on juice strained through three thicknesses of cheesecloth. Exclusion of all pulp may have influenced our results, or the variety of oranges may be the determining factor.

The Hawaiian squash (or pumpkin) had almost three times more thiamine than the reported average. The variety used was not identified botanically but was one favored by the Filipinos in Hawaii and may have been a cross between *Curcubita moschata* and *Curcubita maxima*.

The pear-shaped variety of tomato tested for thiamine contained more than twice the reported average value.

As previously noted (p. 7), breadfruit, taro and poi, potatoes, sweetpotatoes, and squash or pumpkin are all good sources of thiamine when compared on the calorie basis with whole-grain products. Some years ago Williams pointed out that nature has supplied whole grains with adequate thiamine to take care of the metabolism of the carbohydrates which they contain (45). It would seem to be equally true of these foods high in starch and sugar. It might be noted here that the quantity of poi commonly eaten by an adult Hawaiian in ancient times could easily furnish 2 milligrams of thiamine daily. Sweetpotatoes and breadfruit, two other important sources of calories in the old Hawaiian diet, also furnished good quantities of thiamine.

It was assumed that green soybeans, which have long been a favored food of the Oriental peoples, prepared by cooking them in the pods in

boiling salted water, would retain their original thiamine content, but beans tested before and after being cooked in this manner were found to lose almost 40 percent of the original thiamine content (see table 1, p. 19).

Although it is well known that rice polish and rice bran are potent sources of the B vitamins, work from this Station was the first to show that when vegetables are salt-pickled with rice bran in the oriental manner, they adsorb thiamine from the rice bran (21, 27). The vegetables retain the thiamine even though all particles of rice bran are removed by washing the vegetables thoroughly in tap water. The quantity of thiamine adsorbed appears to depend upon the amount and kind of vegetable surface exposed. As a result of pickling three vegetables in salt and rice bran for a period of 3 days, the thiamine content was increased over that originally occurring in the vegetables as follows: Chinese cabbage (*Brassica chinensis*), four times; long eggplant used without peeling, one and a half times; and daikon (long white radish), approximately seven times. On the other hand, pickling with salt alone tended to destroy a portion of the thiamine. Values for the raw and bran-salt-pickled vegetables may be found in table 1. Even though used in smaller amounts than Americans customarily use fresh vegetables, these bran-salt-pickled products that are often used three times a day may make a worth-while contribution of thiamine to the diet. Their preparation should be encouraged in contrast to the "plain salt-pickled ones.

Macadamia nuts, produced commercially in Hawaii, were found to be an excellent source of thiamine.

The thiamine value for raw lean pork (table 1), which is less than that usually reported for grain-fed pork, is probably related to the thiamine content of the ration, since it has been shown by others (32) that the thiamine in pork tissues is directly related to the intake of this vitamin by the pig. The loss as a result of roasting, about 40 percent, is in line with other reports (16, 37, 44).

The average value of 260 micrograms of thiamine per 100 grams of raw rice, calculated from three bio-assays of the cooked product (298, 205, and 280 micrograms), is lower than the average value of 290 micrograms given in Miscellaneous Publication 572, but the two higher values are much the same. Partially polished rice had about 70 percent as much thiamine as the particular lot of brown rice from which it was made. A study of the thiamine content of brown and partially polished rice and the effect of washing the rice before cooking has been reported elsewhere (24).

Employing the bio-assay method used in the Station Laboratory, we could demonstrate no thiamine in cooked white rice which had been washed in four or five changes of tap water prior to cooking, as is customarily done by people of Oriental ancestry in Hawaii.

"Black" rice, which has deep red or purple pigments in the pericarp or outermost layer and a snowy white interior, is a glutinous type of rice imported in small quantities from the Philippines. Its thiamine value is similar to the brown rice samples tested in this laboratory.

ASCORBIC ACID

The values for 31 foods listed in table 1 were compared with the average figures in Miscellaneous Publication 572. Eleven vegetables and two fruits grown in Hawaii with lower than average values were avocado, green beans, beet greens, broccoli, carrots, chard, eggplant, lettuce, okra, green peppers, pineapple, sweetpotatoes, and turnip greens. Five of these—beet greens, broccoli, chard, green pepper, and turnip greens—may be considered important vegetable sources of ascorbic acid, and the published averages are all far above the values found for locally grown products which were obtained from the market. Additional work will be necessary to determine whether these differences are the result of exposure to warm temperatures after harvesting and the lack of proper refrigeration or whether climatic or varietal factors are the controlling ones.

Four Hawaiian-grown foods—celery, orange juice, pumpkin or squash, and strawberries—had higher than average ascorbic acid values.

Fourteen other foods had values within 5 milligrams of the averages given in Miscellaneous Publication 572.

The ascorbic acid value for raw mung bean sprouts (15 milligrams per 100 grams), given in table 1, represents an average for fresh sprouts purchased from the market. When one lot of very fresh sprouts was placed in tap water and stored in the refrigerator for 24 hours, 22 percent of the original ascorbic acid was lost. An additional 24 hours' storage in the refrigerator did not increase the loss, but after 70 hours the loss was 50 percent. When sprouts from the same lot were stored for 24 hours in tap water at room temperature, they lost 44 percent of their ascorbic acid, and after 46 hours at room temperature they had lost all of their original ascorbic acid.

Fresh imported asparagus had only one third the amount of ascorbic acid of locally grown asparagus. Imported carrots and potatoes had slightly lower values than were found for the locally produced foods.

Some Hawaiian-grown foods (not appearing in Miscellaneous Publication 572) which are excellent sources of ascorbic acid are: (eight vegetables) belembe, green mustard cabbage, white mustard cabbage, Chinese cabbage, Malabar nightshade, watercress, and the leafy tender tips of bitter melon and horseradish tree; and (eight fruits) carissa, guava, ketambilla, litchi, longan, some varieties of mango, papaya, and pomelo.

VITAMIN D

Because vitamin D occurs in so few foods (fruits and vegetables contain no vitamin D) and because the need for this vitamin, as explained on page 9, seems less important in a sunny climate like Hawaii, only four foods have been tested—egg yolk, winter and summer milk, opihi (Hawaiian limpet), an important food of the ancient Hawaiians, and canned pilchards. The latter were available in large quantities during the war when the supply of local fish was greatly restricted.

The opihi were tested some years ago as part of a general plan to study

the foods of the ancient Hawaiians. According to our Hawaiian informants, opihi, which in modern times have become a luxury food, were formerly found on all the islands the year round and were eaten by all classes of people. The soft parts of the opihi, mixed with poi, constituted one of the first foods fed to Hawaiian infants in olden days. Opihi are usually eaten raw, either fresh or salted, frequently with certain seaweeds.

Original data on the nutritive value of opihi, including the results of feeding them as the sole source of vitamin D, have been published elsewhere (30). To test the vitamin D value, opihi were fed in five forms—whole; viscera or total soft parts; viscera without the gonads; testes; and ovaries. The results proved that the soft parts had four times as much vitamin D as the whole opihi, that the two kinds of gonads had essentially the same amount, and that both gonads contained somewhat more of the vitamin than the viscera alone (see table 2).

TABLE 2. Vitamin D values of foods.

FOOD	I. U./100 GM.
Egg yolk	200
Milk, summer	4
Milk, winter	2
Opihi, whole	50
Opihi, viscera	200
Pilchards	1,300

The results of feeding milk produced in Hawaii as the sole source of vitamin D have been published elsewhere (26). Winter milk was found to have approximately half the vitamin D value of summer milk (table 2), and both values were similar to those for milk produced elsewhere.

Cooked egg yolk, from eggs produced on the University Farm, was fed to rachitic rats at two levels, 0.25 gram and 0.5 gram daily. Suitable negative and positive controls were provided in the usual way. The degree of healing for a group of 10 rats fed 0.25 gram of egg yolk daily was the same as that of the rats fed 0.5 I. U. of vitamin D from the reference cod liver oil. It was calculated that 100 grams of egg yolk contained 200 I. U. of vitamin D, a value within the range of those reported for average eggs (19).

On the basis of the experiments just reported it may be calculated that 1 quart of milk may contain 20 to 40 I. U. of vitamin D and one egg yolk, weighing 15 grams, 30 I. U. The National Research Council allowance for infants up to 1 year of age is 400 to 800 I. U. vitamin D. Jeans (17) has recommended that babies receive at least 350 I. U. vitamin D daily—a quantity which will not only prevent rickets but will also assure good bone and tooth calcification throughout the growth period. It is obvious that a quart of milk and an egg yolk will supply only a small portion of the daily need for this vitamin, and that regular exposure to sunshine or some extra source of vitamin D is needed.

The California canned pilchards (a herring-like fish) tested were packed with salt and water. The water was drained off, the bones were removed, and the flesh was thoroughly mixed before feeding. The average rate of healing for nine rats was 3.6+ when 0.1 gram was fed daily. Compared with the positive controls and the curve of response, the pilchards were calculated to contain 1,300 I. U. of vitamin D per 100 grams. The same value has been reported for sardines (11). Such fish are valuable sources of vitamin D, as one small fish about 4 inches in length will furnish the amount of vitamin D recommended for a growing child or a pregnant woman (36).

SPECIAL STUDIES OF ASCORBIC ACID VALUES

The chemical method of determining ascorbic acid made it possible to test a larger number of foods than were tested biologically for vitamin A and thiamine and to make some detailed studies of factors, such as variety, cooking processes, and storage, which affect the amount of ascorbic acid in foods.

EFFECT OF VARIETY UPON THE ASCORBIC ACID CONTENT OF FRUITS

Data in table 3 show the ascorbic acid values of different varieties of avocado, banana, guava, lime, and mango.

More work is needed on avocados and bananas, but the values obtained to date indicate that some varieties have two or three times more ascorbic acid than others. The relatively low values for Bluefield and Chinese bananas have been substantiated by bio-assay. These two are the most important local commercial varieties.

Common guavas growing wild in Hawaii exhibit a wide range of ascorbic acid values. They also vary in acidity, shape, and color. Under cultivated conditions, a number of them would probably be described as horticultural varieties. Some of them are sweet, some sour, and some semi-sweet. The lemon-shaped guava is the most common but others are round and a few are pear-shaped. Although all have a light yellow rind, the color of the interior varies from almost white through shades of yellow and pale pink to deep pink. All the wild guavas tested have been found to be good sources of ascorbic acid but since the highest values are about five times the lowest, selection and propagation of the better varieties might yield fruit with even higher values.

The pale yellow Cattley guavas tested thus far have shown higher values than the red or strawberry guavas.

Four varieties of limes listed in table 3 have ascorbic acid values ranging from 10 to 30 milligrams per 100 milliliters of juice. Limes are obviously less potent sources of this vitamin than other citrus fruits.

This Station has previously reported variations in the ascorbic acid content of mangoes grown in Hawaii (10). Thirty-four varieties listed in table 3 show a wide range of ascorbic acid values, from 5 to 142 milligrams per 100 grams. Although there are doubtless variations in individual mangoes from the same tree, as well as differences from season to season,

TABLE 3. Effect of variety upon the ascorbic acid content of fruits.

NAME AND VARIETY	ASCORBIC ACID	NAME AND VARIETY	ASCORBIC ACID
	<i>Milligrams/100 grams</i>		<i>Milligrams/100 grams</i>
Avocado		Mango (ripe)	
West Indian race		Accession No. 1975	50
Purple rind		Bishop	33
No. 1	5	Bombay yellow	5
No. 2	12	Borsha	15
Green rind		Brooks Late	28
No. 1	5	Cambodiana	35
No. 2	9	Cigar	119
No. 3	16	Common (6 samples, 3 seasons)	
No. 4	15	(Manini)	70 to 142
Guatemalan		Fairchild (3 samples)	18 to 23
No. 1	8	Goa Alphonse	43
Banana		Haden (5 samples)	13 to 17
Bluefield	6	Holt	44
Brazilian	16	Indian Race	56
Chinese	8	Itamaracca (average, 2 seasons)	45
Ice Cream	19	Julie	50
Popoula Kaio (cooking banana)	15	Kruse	20
Guava		Lemon Chutney	31
Common, ripe (100 samples)	70 to 350	Moreland	35
Cattley		Number 9	37
Red	33	Philippine type	15
Yellow	53	Pirie (6 samples, 3 seasons)	14
Lime (juice)			(range 12 to 16)
Barss	24	Pirie, Koboni	13
Kusaie	19	Pirie, Jordan	26
Lakeland	30	Pirie seedling	28
Rangpur	10	Robinson	21
		Sandersha	26
		Seedlings	
		1	34
		2	37
		3	92
		4	97
		Smith-Wooten	80
		Strawberry	24
		Wilcox	11
		Wooten	63 to 90

enough has been done on some of the varieties to show that true varietal differences of considerable magnitude do exist. For example, samples of the common mango (sometimes called the Manini) from different sources and seasons show considerable variation. The values are relatively high—70 to 142—in comparison with the true Pirie. Samples of the Pirie from different sources during three seasons showed consistently low values of from 12 to 16 milligrams of ascorbic acid per 100 grams.

Since the values given in table 3 were determined, Munsell has also reported wide variations in the ascorbic acid of mangoes from Puerto Rico (34).

CHANGES IN THE ASCORBIC ACID CONTENT OF FRUIT DURING RIPENING

When mangoes were being tested for their ascorbic acid values it soon became evident that the degree of ripeness affected the quantity of the vitamin present. Data for the ascorbic acid of 14 varieties at two or at three stages of ripeness are given in table 4. As the samples in each case consisted of sections from four to six mangoes from the same tree, the values are considered to be true differences resulting from the stage of ripeness and not differences in individual mangoes. In every instance the quantity of ascorbic acid decreased as the mangoes ripened.

Unlike mangoes, papayas and pohas show an increase of ascorbic acid as the fruit ripens, as indicated by the data in table 4.

TABLE 4. Changes in ascorbic acid content of fruit during ripening.

FRUIT	VERY GREEN	GREEN	HALF-RIPE	RIPE
	Mg./100 gm.	Mg./100 gm.	Mg./100 gm.	Mg./100 gm.
Mango				
Cigar	---	---	154	119
Common (Manini)	---	188	145	114
Fairchild	---	---	31	19
Haden	---	42	---	14
Indian race	---	---	61	56
Itamaracca	---	---	53	40
Number 9	---	43	37	30
Philippine type	---	---	25	15
Pirie	---	60	50	14
Pirie seedling	---	---	60	28
Sandersha	---	---	33	26
Smith-Wooten	---	---	105	79
Strawberry	---	42	---	24
Wooten	---	103	---	63
Papaya				
Series 1 (large-fruited type)	32	40	53	68
Series 2 (Solo variety)	31	72	95	102
Poha	---	31	36	42

ASCORBIC ACID CONTENT OF GUAVAS

In addition to the studies reported in table 3, other studies have been made to determine the distribution of ascorbic acid in the two parts of the guava and losses of ascorbic acid during preparation and storage of juice.

Distribution of ascorbic acid in rind and pulp of guavas

The thick rind portion of the common guava contains more ascorbic acid than the pulp and seeds, both because there is a greater proportion of the rind than pulp in each guava, and because per unit of weight the rind is richer in ascorbic acid. The thick rind makes up about 60 percent of the weight and the pulp about 40 percent. Four sets of guavas were weighed and assayed. The ascorbic acid values and the percentage distribution of the vitamin between rind and pulp were found to be as follows:

WHOLE GUAVA	RIND		PULP	
Mg. /100 gm.	Mg. /100 gm.	Percent	Mg. /100 gm.	Percent
306	326	66	276	34
134	165	74	87	26
116	131	70	86	30
107	129	70	84	30

Losses of ascorbic acid in the preparation of guava juice

Sampling procedure.—To determine the most satisfactory method of sampling guavas, a slice or wedge was removed from each of five guavas. Extracts were made from the slices or wedges and also from the remaining portion of each guava. The results were as follows:

	Mg. /100 gm.
1. Equatorial section	85
Remainder	101
2. Equatorial section	82
Remainder	93
3. Longitudinal wedge	114
Remainder	114
4. One quarter	297
Remaining three quarters	305
5. One quarter	262
Remaining three quarters	257

The results indicate that a more representative sample can be obtained by using a wedge or quarter than an equatorial section.

Effect of standing.—To check on possible losses due to oxidation during preparation and standing, a simple experiment with four guavas was used. One quarter was removed from each, weighed, and extracted at once. A second quarter of each guava was weighed, chopped, and allowed to stand at room temperature for $3\frac{1}{2}$ hours before extracting. The following ascorbic acid values were obtained:

GUAVA	TIME OF STANDING		LOSS
	None	$3\frac{1}{2}$ hours	
	Mg. /100 gm.	Mg. /100 gm.	Percent
1	348	270	22
2	122	64	48
3	103	74	28
4	248	204	18

The losses of ascorbic acid were fairly large and also variable, and even though the time of standing was much longer than would ordinarily occur in practice, it is apparent that cut guavas should not be allowed to stand at room temperature.

Ascorbic acid of guava juice and the remaining pulp.—Ten guavas were used in each experiment. Longitudinal wedges were removed from each guava, weighed, and assayed individually. The remaining portions, which were used to prepare the juice, were weighed individually and the amount of ascorbic acid present was calculated on the basis of the portion assayed. The sum of these values was used as the total milligrams of ascorbic acid in the raw sample.

To prepare the juice (literally a watery extract of the guavas) the guavas were sliced quickly, covered with water in a large glass beaker, and

boiled for 15 minutes. The mixture was weighed, a sample removed for assay, and the remainder filtered through cheesecloth. The pulp and the juice were weighed and assayed. The results of two trials are given below:

ITEM	WEIGHT	ASCORBIC ACID CONTENT		
		Per 100 gm.	Total	Percent of Total
<i>Trial 1</i>	<i>Gm.</i>	<i>Mg.</i>	<i>Mg.</i>	
Raw guavas	518	---	648	100
Cooked guavas plus water	845	78	660	102
Juice	471	74	348	54
Pulp	293	61	178	27
Juice plus pulp	---	---	526	81
Loss in preparation	---	---	---	19
<i>Trial 2</i>				
Raw guavas	587	---	548	100
Cooked guavas plus water	907	54	490	89
Juice	621	53	329	60
Pulp	259	41	106	19
Juice plus pulp	---	---	431	79
Loss in preparation	---	---	---	21

The tabulated data indicate that 50 to 60 percent of the ascorbic acid of guavas is extracted in the juice prepared by this method and that approximately 20 to 25 percent remains in the pulp. The remaining pulp is a relatively good source of ascorbic acid as weight for weight it contains approximately 80 percent as much ascorbic acid as the juice.

The loss of about 20 percent of the ascorbic acid may be due partly to enzymic oxidation prior to heating and partly to auto-oxidation during the cooking and straining processes.

Effect of storage on the ascorbic acid content of bottled guava juice

The watery extract of guavas, here called guava juice, makes a fine fruit drink that is an excellent source of ascorbic acid. To determine the stability of the vitamin in homemade guava juice, two series of experiments, each of 11 months' duration, were carried out. The juice was bottled in clear and brown glass bottles, sealed with crown-type caps, stored in a dark cupboard at room temperature, and tested monthly.

For the first series, a sufficient quantity of guava juice to fill 24 bottles was brought to a boil in a large kettle and kept at the simmering point while two people filled and capped the bottles as rapidly as possible. When the first bottles were tested, it was apparent that the evaporation which took place during the bottling process resulted in an increasing amount of ascorbic acid in each successive bottle filled, rather than some destruction as might have been expected. However, the juice from one brown bottle and one clear bottle was tested each month for its ascorbic acid content. It was evident that the ascorbic acid decreased on storage, and it was calculated that in 11 months there was a loss of approximately 30 percent.

To overcome the variation in ascorbic acid values between bottles caused by evaporation during heating of the juice, a different method of bottling was used for the second series. A large volume of juice was prepared as before, and from this were taken volumes sufficient to fill two bottles. These small volumes were then brought to boiling under uniform conditions with respect to rate and time of heating. One clear and one brown bottle were filled each time, with the order of filling (with respect to brown or clear glass) alternating with each pair.

The data in table 5 on the ascorbic acid content of this bottled guava juice, stored in the same manner as the first series, show a loss of approximately 30 percent in 11 months for both types of bottle. It is believed that the low value for the juice in the brown bottle tested after 10 months of storage was caused by a poor seal, as it is much out of line with the other figures.

TABLE 5. Effect of storage on the ascorbic acid content of bottled guava juice.

MONTHS OF STORAGE	ASCORBIC ACID PER 100 MILLILITERS OF JUICE		
	Bottles		Average
	<i>Brown</i>	<i>Clear</i>	
0	83	88	86
1	85	77	81
2	81	75	78
3	69	75	72
4	69	66	68
5	68	76	72
6	68	72	70
7	62	67	65
8	63	69	66
9	59	60	60
10	43 (?)	58	51 (?)
11	59	62	61

Despite its high acidity (pH 3.5), guava juice, properly bottled, sealed, and stored, gradually decreases in vitamin C potency, although even at the end of a storage period of 11 months it may be as good a source of ascorbic acid as average orange juice.

It was at first assumed that such strongly acid juice could be stored (after opening) in the refrigerator for some weeks without any marked change in the ascorbic acid content. However, some tests during the first series indicated a considerable loss in 2 weeks and a serious loss in 4 weeks. To test this point carefully, 14 of the samples used for the tests recorded in table 5 were tested weekly for 5 weeks and the results are summarized in table 6. It is obvious that guava juice once opened should be used at once or within a period of 2 weeks. The flavor and color of the juice do not seem to be impaired after even 4 or 5 weeks of storage, but the ascorbic acid is quite certain to have been destroyed.

No dehydroascorbic acid could be detected in these samples by the usual chemical method using reduction by hydrogen sulfide. This fact indicates that the juice no longer had any value as a source of ascorbic acid. This was further confirmed by giving large quantities of the juice to two

human subjects who had been receiving a constant intake of 75 milligrams of ascorbic acid per day for 5 weeks and had reached a fairly uniform level of excretion. The subjects received the guava juice for a period of 1 week during which time the rate of urinary excretion decreased sharply and at a rate comparable to that of a third subject who received no guava juice during this week.³

TABLE 6. Loss in ascorbic acid content of bottled guava juice stored in refrigerator after opening.

Weeks of storage in refrigerator	14 SAMPLES	
	AVERAGE	PERCENT LOSS
	Mg./100 ml.	
0	64	---
1	56	12
2	43	33
3	26	59
4	8	88
5	1	98

Ascorbic acid values of typical home-canned and bottled guava juice and pulp

Home preparation of guava products, considered a good practice in normal times, was especially recommended as a part of the food conservation program during the war. Work carried on in the Station had proved that guava products prepared in the laboratory were excellent sources of ascorbic acid but whether typical home-canned and bottled guava juice and pulp were equally good was uncertain.

Fifty samples of home-canned guava products—14 of guava pulp, 6 of pulpy juice, and 30 of strained juice—were collected from different localities on four islands.⁴ Many of the samples were tested 3 and 4 months after bottling; and, as the containers were not always of the best type, some deterioration in the ascorbic acid values could be expected, judging from the work reported on page 29.

The samples varied in their ascorbic acid content from 32 to 130 milligrams per 100 milliliters, with an average of 66 milligrams. As most of the values were between 40 and 110 milligrams, the products were equal to (or superior to) orange juice as a source of ascorbic acid. In everyday terms the day's quota of ascorbic acid may easily be obtained from 1/3 to 2/3 of a cup of home-prepared guava juice or pulp.

³ Personal communication from Eva Hartzler, Associate Nutritionist, Hawaii Agricultural Experiment Station.

⁴ Many of the samples were obtained by the home demonstration agents of the University Agricultural Extension Service through the courtesy of K. Shellhorn, Assistant Director.

ASCORBIC ACID CONTENT OF MANGOES

Effect of method of preparation and storage (in refrigerator)

To test the stability of the ascorbic acid in mangoes, samples were prepared from three ripe and from three half-ripe to green fruits. The mangoes were peeled and the cheeks removed from each side. Half of each sample was assayed at once and the other half allowed to stand on a watch glass at room temperature in the laboratory for 6 hours. The green mango sample assayed at once contained 61 milligrams of ascorbic acid per 100 grams, and the sample which stood for 6 hours contained 59 milligrams. The fresh ripe sample contained 44 milligrams, and after standing, 42 milligrams. In view of the difficulty in getting exact duplicate samples from mangoes, and the fact that the difference in values is less than 5 percent, the loss may be considered negligible.

To determine the effect, upon the ascorbic acid content, of cooking mangoes in glass and aluminum containers in family-sized lots (4 cups), half-ripe mangoes were prepared, sampled, and assayed prior to cooking. Weights of the mangoes, before and after being cooked with appropriate amounts of water, were recorded and the equivalent weights of raw and cooked mangoes were determined. A wooden spoon was used for stirring the mangoes in the glass container and an aluminum spoon in the aluminum saucepan. No losses were found as a result of cooking.

To study further the effect of the method of preparation upon the ascorbic acid in mangoes and to determine the losses during storage in the refrigerator, two large lots, A and B, of half-ripe mangoes were prepared. Each lot was then further divided into two portions, making a total of four. Each lot was cooked in the same type and size of aluminum saucepan but the spoons and strainers used for stirring and sieving were different. Lot A was prepared without sugar and lot B with sugar. All lots were stored, without sterilizing or sealing, in glass jars with Bakelite covers in an electric refrigerator for the periods indicated. The results are summarized in table form on page 32.

After 35 days of storage all samples of the mango sauce showed signs of molds. The presence of sugar in the sauce seemed to have little or no effect upon the ascorbic acid content. Sauce prepared in this manner would normally not be stored without processing for a period longer than a week. The data given here indicate that the loss for such storage should not exceed 10 percent.

The losses of ascorbic acid when the sauce was prepared by using a worn metal spoon (unknown alloy) and an old wire strainer showing signs of corrosion and rust were less than anticipated. They did not greatly exceed those of mango sauce prepared in an aluminum container with a wooden spoon, which was previously shown to preserve the ascorbic acid as well as a glass container.

All of our experiments to date indicate that the ascorbic acid in mangoes is relatively stable.

DAYS OF STORAGE	LOT A, NUMBER 1, WOODEN SPOON AND ALUMINUM STRAINER		LOT A, NUMBER 2, METAL SPOON AND OLD WIRE STRAINER	
	Ascorbic acid	Retention	Ascorbic acid	Retention
	Mg. /100 gm.	Percent	Mg. /100 gm.	Percent
0	44	---	41	---
8	39	89	35	85
14	35	80	31	75
21	34	77	29	71
35	33	75	24	59

DAYS OF STORAGE	LOT B, NUMBER 1, SAME AS LOT A, NUMBER 1, PLUS SUGAR		LOT B, NUMBER 2, SAME AS LOT A, NUMBER 2, PLUS SUGAR	
	Ascorbic acid	Retention	Ascorbic acid	Retention
	Mg. /100 gm.	Percent	Mg. /100 gm.	Percent
0	39	---	37	---
8	36	92	33	89
14	34	87	30	81
21	31	80	26	70
35	30	77	25	68

ASCORBIC ACID CONTENT OF PAPAYAS

Stability of ascorbic acid in papaya

To test the stability of the ascorbic acid in papaya, lengthwise sections were taken from one papaya. The portion assayed at once had an ascorbic acid value of 96 milligrams per 100 grams. After standing on a watch glass at room temperature in the laboratory for 2½ hours, a portion was assayed and found to contain 99 milligrams of ascorbic acid per 100 grams. These figures are within the range of values likely to be found in sampling any one fruit.

Variations in the ascorbic acid content of papaya

Fruits were collected at weekly intervals over a period of 1 year, except for the month of August, from Poamoho Experimental Farm and from Kailua. Forty-five papayas from Poamoho contained an average of 81 ± 1.7 milligrams of ascorbic acid per 100 grams of edible papaya (range 60 to 122 mg.), and 40 fruits from Kailua contained an average of 88 ± 2.5 milligrams (range 59 to 118 mg.). When the values were plotted for the year, the highest values were found to occur in April, May, June, and October.

Additional experiments are necessary to determine the factors that affect the ascorbic acid values and to learn if they are correlated with the sugar content. The Plant Physiology Department has determined that the sugar content of papaya fruit is correlated with sunlight and is highest during the months of May to October inclusive.⁵

⁵ Personal communication from Dr. R. C. Lindner, Plant Physiology Department, Hawaii Agricultural Experiment Station.

Ascorbic acid content of papaya seeds

One sample of fresh papaya seeds, including the aril, was tested for vitamin C and found to contain 78 milligrams ascorbic acid per 100 grams of seed; the papaya from which the seeds were taken had a value of 93 milligrams ascorbic acid.

Two samples of the aril (the fleshy material surrounding the black seeds) were tested and found to have 130 and 161 milligrams ascorbic acid per 100 grams; the fruits from which these samples were taken contained 72 and 98 milligrams, respectively.

Effect of cooking upon the ascorbic acid content of papaya

To determine the loss of vitamin C in papaya due to cooking, the ascorbic acid contents of two samples of papaya in the fresh state, one from Kailua and one from Poamoho, were compared with those of half of the same papayas baked in an electric oven at 350° F. for 20 to 25 minutes. One sample lost 7 percent and the other, 12 percent. Cooking directions often call for the addition of lemon or lime juice, which will increase the acidity and probably improve the retention of ascorbic acid in heated papaya. The pH value for three samples of papaya was found to be 6.0.

A sauce made by simmering papaya with a little sugar for 20 minutes lost 7 percent of the original ascorbic acid.

All experiments to date indicate that the ascorbic acid in papaya exists in a very stable form.

ASCORBIC ACID CONTENT OF COMMERCIAL FRUIT JUICES AND NECTARS

Twenty-one samples of canned and bottled Hawaiian fruit juices and nectars have been assayed for their ascorbic acid content. The results are summarized in table 7.

It may be noted that there is a wide range of values for each type of juice. The exact methods used in preparing these commercial products are

TABLE 7. Ascorbic acid values of commercial fruit juices and nectars.

PRODUCT	MILLIGRAMS PER 100 GRAMS	PRODUCT	MILLIGRAMS PER 100 GRAMS
Banana nectar	0	Guava nectar	
Guava juice		Brand 5 (thick)	66
Brand 1	32	5 (thick)	50
2	45	5 (thick)	74
3	25	6	10
4	19	Papaya juice and nectars	
5	4	Brand 1	10
Guava nectar		2	20
Brand 1	3	3	14
2	11	4	22
3	16	5	81
4	20	6	12
		6	39

not known, but it is believed that the relatively low values found for most of them are the result of several factors, namely, (1) improper handling of the fresh fruit, (2) contact with metals, especially copper retorts and pipes, (3) heating and holding in contact with oxygen of the air, and (4) dilution with water.

Commercial methods of preparing and processing guavas and papayas, which will preserve the original ascorbic acid content to a much greater degree, can doubtless be devised. Establishment of government specifications for locally produced fruit drinks would make it possible to purchase commercial Hawaiian fruit juice and nectars guaranteed to contain not less than specified amounts of ascorbic acid. At the present time (1946) they cannot be depended upon as sources of vitamin C.

ASCORBIC ACID CONTENT OF JAMS AND JELLIES

The loss of ascorbic acid in making guava jelly has been found to be very small, but the loss during storage is marked. One hundred grams of guava juice, containing 68 milligrams of ascorbic acid, yielded 148 grams of jelly containing 65 milligrams of ascorbic acid. After storage for 3 months in a dark cupboard, this same jelly had an ascorbic acid content of 16 milligrams per 148 grams. That stored on a shelf in a well-lighted laboratory (but not in direct sunlight) also contained 16 milligrams. Under both conditions the loss of ascorbic acid in guava jelly was about 75 percent.

Eight other samples of jellies and jams were assayed within a day or two after preparation (table 8) and a second previously unopened sample of each was again assayed after being stored, away from light at room temperature, for 6 months. All samples were in clear glass containers, sealed with paraffin only, and covered with papers. It was found that 50 to 80 percent of the original ascorbic acid had been lost.

In Hawaii, where fresh fruits and vegetables that are good sources of

TABLE 8. Ascorbic acid values of homemade jams and jellies (freshly prepared).

PRODUCT*	MG./100 GM.
Carissa jelly	20
Guava butter	80
Guava jelly	53
Guava marmalade	32
Guava-papaya jam	32
Java plum and guava jelly	35
Papaya marmalade	37
Papaya-pineapple jam	25
Ketambilla-papaya jam	54
Ketambilla-guava jelly	39
Ketambilla-apple butter	29
Roselle jelly	2

* All products were made according to recipes in Fruits of Hawaii, University of Hawaii Agricultural Experiment Station Bulletin 96, except papaya-pineapple jam, which contained fruit in the proportions of 7 cups of papaya to 4 cups of pineapple.

ascorbic acid are available the year round, the contribution which jams and jellies are likely to make to the day's need for this vitamin is small. In areas where fresh fruits and vegetables are not available throughout the year, preserved foods may be the main source of vitamin C. Consequently, since this preliminary experiment indicates large losses of ascorbic acid in 3 months, additional work is required to determine if and in what way the losses can be reduced.

CHANGES IN THE ASCORBIC ACID OF STORED POTATOES

Bliss Triumph variety of potatoes (known locally as Hawaiian Rose), assayed within 3 to 5 days after harvest, was found to have much higher ascorbic acid values than are commonly given for potatoes in compiled tables of average vitamin values. Consequently, the potatoes were stored for 2 months and again tested for ascorbic acid. They were then found to have a value similar to that usually reported.

In Hawaii, potatoes are normally stored only for short periods, because of the great demand for new potatoes during the months of January to March inclusive, when locally produced potatoes are usually available. However, to determine the effects of some weeks of storage at room temperature on the ascorbic acid content, potatoes were tested at bi-weekly intervals over a period of 12 weeks. The vitamin content decreased gradually over a period of 8 weeks. By the last week the potatoes were beginning to spoil, yet the ascorbic acid content was as great as after 8 weeks of storage.

It is apparent from the data in table 1 and table 9 that new potatoes may constitute a good source of ascorbic acid in the diet even after a storage period of 4 weeks.

TABLE 9. Changes in the ascorbic acid content of Bliss Triumph (Hawaiian Rose) potatoes on storage.

STORAGE	AVERAGE ASCORBIC ACID CONTENT	LOSS
<i>Weeks</i>	<i>Milligrams per 100 grams</i>	<i>Percent</i>
0	35	0
2	29	17
4	26	26
6	20	43
8	17	51
10	17	51
12	17	51

SUMMARY AND CONCLUSIONS

Vitamin A values for 85 foods, thiamine values for 90 foods, ascorbic acid values for more than 150 foods, and vitamin D values for 4 foods are reported in this bulletin. These foods include some of tropical and semi-tropical origin and some characteristic of the diets of the racial groups living in the Islands, as well as common American foods produced in Hawaii.

Chemical and biological methods of vitamin assays, as well as the preparation of the foods for assay, are described.

If average values for similar foods appear in U.S.D.A. Miscellaneous Publication 572, they have been compared with the Hawaii values.

Conclusions may be briefly summarized as follows:

A number of Hawaii foods, not listed in Miscellaneous Publication 572, were found to be excellent sources of vitamin A, thiamine, and ascorbic acid.

The vitamin A values of practically all the Hawaii foods tested are higher than average.

With few exceptions, the thiamine contents of Hawaii foods are similar to the published values.

A number of Hawaiian-grown vegetables purchased on the market have lower than average ascorbic acid values.

Milk and eggs produced in Hawaii contain about the same amount of vitamin D as those produced elsewhere.

Opihi, a Hawaiian shellfish, are a fair source of vitamin D and canned pilchards a good source.

Avocados, bananas, guavas, limes, and mangoes show marked varietal differences in ascorbic acid values.

Mangoes decrease in ascorbic acid content as the fruit ripens, whereas papaya and poha show increased ascorbic acid as the fruit ripens.

Bottled guava juice kept in the dark at room temperature loses 30 percent of its original ascorbic acid value in 11 months' storage.

Unsealed guava juice stored in a refrigerator loses practically 100 percent of its ascorbic acid in 4 weeks' storage.

The ascorbic acid in mangoes and papayas is relatively stable, and little loss occurs on standing or during cooking.

Commercially canned fruit juices and nectars containing papaya and guava are undependable sources of ascorbic acid.

Bliss Triumph variety of potatoes, a good source of ascorbic acid when new, loses vitamin C progressively for 8 weeks when stored for a period of 12 weeks. The losses do not increase from 8 to 12 weeks.

APPENDIX I

BIOLOGICAL DETERMINATION OF VITAMIN A

CURVE OF RESPONSE

The determination of the vitamin A values of food by bio-assay is beset by many difficulties, and various modifications of the growth response method as well as other techniques have been proposed (4, 6, 8, 40, 43) without arriving at one that is generally accepted or that proves satisfactory in all laboratories.

Procedures recommended by Swanson *et al.* (43) and by Coward (6) were of great aid in establishing the practices in the Station laboratory.

In the hope of determining a good basis for our assays, the procedure outlined here was used to determine a curve of response.

Methods

The diet fed to the mothers of the experimental rats during breeding and the first 10 days of the lactation period has been reported elsewhere (22). Ten days after the birth of the rats, the mothers and young were given the following diet: whole wheat, 1,175 grams; skim milk powder, 400 grams; and sodium chloride, 10 grams. The young were weaned at 17 or 18 days of age and continued on the restricted diet until 21 days of age.

At 21 days of age the rats were placed on the following experimental diet: vitamin A-free casein, 18 percent; dried yeast, 10 percent; salt mixture,¹ 2.5 percent; sodium chloride, 1 percent; cornstarch, 58.5 percent; hydrogenated fat (Crisco), 10 percent; sufficient viosterol in cottonseed oil (Wesson) to furnish 0.5 to 0.6 I.U. of vitamin D per gram of basal diet. The reference cod liver oil,² used to establish the curve of response and thereafter for comparison with the natural foods, was first prepared for feeding as follows: The specific gravity of Wesson oil that had been saturated with dry CO₂ for 5 hours was determined and used to dilute the reference cod liver oil so that 0.1 milliliter equaled 3 International Units. Later the reference oil was weighed in a volumetric flask and made to volume with Wesson oil. Feeding tests of the Wesson oil proved it to be devoid of vitamin A.

Supplements were planned to supply the following daily quantities of vitamin A: 0.5, 1, 2, 3, 4, and 8 I.U. They were fed twice a week for the first three quantities, three times for 3 International Units, four times for 4, and five times for 8. The supplements, fed in glass castor cups, were delivered from a calibrated microburette or an "Agla" micrometer syringe.

For judging the exhaustion of vitamin A stores of the rats, the criteria recommended by Swanson and co-workers were used (43).

¹ Literature Cited, references 15 and 20.

² Purchased from the Committee of Revision of the Pharmacopoeia of the United States.

Results

The average weight of the 182 rats used for the curve of response when placed on the experimental diet was 37 ± 0.3 grams with a standard deviation of 4.0. Swanson *et al.* (43) report a mean weight at 21 days of 43.5 grams with a standard deviation of 14.5 grams for 557 female rats used in their experiments. It is obvious that at weaning our rats were of much more uniform weight than the rats of their colony.

The average period of depletion, 19 ± 2.5 days, was the same for the male and female rats.

Although the weights of the rats at weaning were very uniform, the weights at the end of the depletion period varied greatly. Mean weights and the standard deviations were as follows: males, 100 ± 16.4 , and females, 88 ± 12.3 .

Only one male rat failed to live 3 weeks after supplement feeding was begun; two others failed to live throughout the 5-week feeding period. These were replaced by other rats to make up the required number in each lot. All female rats survived the 3-week feeding period, and only one failed to survive the 5-week feeding period.

In table A are summarized data on the average weights of male and of female rats at weaning and at depletion, and the mean gains (with standard errors) after 3 and after 5 weeks of feeding vitamin A at six levels. Mean weights at weaning and depletion are also given for the negative controls.

The "t" values for the mean gains which were calculated for all groups were highly significant, but those for the 5-week feeding period were not superior to those for the 3-week period.

By using the mean gains given in table A (but omitting the gains for rats fed 8 I.U. daily), curves of response to vitamin A for males and for females were calculated and are shown in figure 1.

It is obvious that the curves level off rapidly. Therefore, for greater accuracy, it is advisable to use only the smaller vitamin A supplements. Signs of vitamin A deficiency are likely to appear in these rats despite gains in weight. A point in favor of a 3-week feeding period is that rats which show only slight signs of a deficiency in 3 weeks show more marked signs if continued for 5 weeks with a resulting loss in rate of gain.

Because the average weights for groups of female rats showed such small increases as the doses of vitamin A were increased, a second series of female rats was fed reference cod liver oil as a source of vitamin A. Thirteen rats were used in each group with proper distribution of the litters between groups. When the daily dosage was at the rate of 1.5, 2, 3, and 4 I.U. of vitamin A daily, the corresponding mean gains in 3 weeks were 50, 51, 57, and 61 grams, increments similar to those previously found.

The differences between the mean gains of the groups of rats receiving different vitamin A supplements, the standard errors of the differences, and the "t" values are presented in table B. The differences in mean gains are significant only for the three lowest levels of vitamin A fed for both males and females. No greater precision was obtained by running the tests

for 5 weeks than for 3 weeks. The curve of response shown in figure 1 indicates that male rats fed 0.5 to 3 I.U. can be used for reference, but for females the curve is good only up to 2 I.U.

The minimum number of rats that must be used to obtain reliable results when natural foods are being tested is extremely important in routine assay work. To obtain an accurate estimate of the error variance of gain in weight of individual rats after supplemental feeding of vitamin A, the methods for the analysis of variance were applied, separate analyses being made for males and females for the 3-week feeding period. The standard errors for gain in weight of a single rat are 9.6 for males and 7.5 for females. Summary of the analysis of variance is presented in table C.³

By using the data in tables A, B, and C, it may be calculated that to demonstrate a difference of 20 percent in gains in weight (odds 30:1) seven male or seven female rats are required.⁴

However, the growth response of male rats to increased quantities of vitamin A is greater than that of females, which means that fewer male rats may be used to demonstrate differences in the vitamin A content of food.

The weight gains of male rats fed levels of vitamin A below 2 I.U. are nearly proportional to the amount of vitamin A fed. Therefore, to detect differences of 20 percent in the vitamin A content of foods, seven or eight male rats may be used.

Even at levels below 2 I.U. of vitamin A the weight gains of female rats are so small that a 20 percent increase in vitamin A results in only about 10 percent gain in weight. Consequently, 25 female rats would be required to detect a 20 percent difference in vitamin A. When seven or eight female rats are used, differences of 50 percent in vitamin A content are all that can be detected.

After completion of the vitamin A experiments with foods and the reference cod liver oil, the gains in weight of all rats used as positive controls and fed 1.5 I.U. daily of reference cod liver oil were pooled and used for the analysis of variance summarized in table D. The mean gains of the groups of male and of female rats that were used as positive controls for each set of experiments over a period of almost 3 years may be found in table E.

From the data in table D, it may be calculated (in the same manner as illustrated in the footnote below) that 12 male rats would be required to demonstrate a difference of 20 percent gain in weight or of vitamin A. Nine females would be required for a 20 percent difference in gain in

³ The authors are indebted to Dr. W. C. Kelly of the United States Plant, Soil, and Nutrition Laboratory at Ithaca, New York, for the statistical analysis of the data and its interpretation.

⁴ Example. For males the mean gain is 59 ± 9.62 . The standard deviation is 16.40 percent of the mean. The probable error of the mean is 11.06 percent. Therefore, to demonstrate a difference of 20 percent in gains in weight (odds 30:1), seven rats are required. See table in J. R. Livermore's *Laboratory Exercises in Statistical Methods of Analysis*, Cornell University, 1941.

weight, but this would detect about 50 percent difference in vitamin A content.

It is to be expected that even if there were no changes in the vitamin A content of the reference cod liver oil during a period of 3 years, the standard error of the mean gain in weight of rats would be greater for this period (table D) than for groups of rats fed at the same time and under the same conditions (table C).

Our rat colony is not kept in an air-conditioned room and although changes in season in Hawaii result in only small changes in temperature and humidity they, along with other unknown factors, undoubtedly affect the growth response of the animals.

Coward (6) has pointed out that there are inherent biological differences in rats and therefore differences in the growth response of even an inbred colony that cannot be accounted for.

Coward has stated that the accuracy of the biological assays for vitamin A obtainable in her laboratory is about 20 percent. On the basis of the statistical analyses just given, the accuracy of the biological method we have used is also about 20 percent, and practically it has been found possible to repeat assays on foods that could be stored with little or no loss of vitamin A and obtain results that checked within 20 to 25 percent.

Summary and conclusions

1. To determine the growth response of rats to graded doses of the vitamin A of reference cod liver oil, 182 rats were given a vitamin A-free diet at 21 days of age; at depletion of vitamin A stores, after about 19 days, 26 rats were used as controls and 156 rats were fed daily doses of reference cod liver oil at six levels (0.5, 1, 2, 3, 4, and 8 I.U.) for a period of 5 weeks.
2. Statistical analysis of the results of a 5-week feeding period and those of a 3-week feeding period showed that the shorter period gives as significant results as the longer period.
3. Curves of response relating the doses of vitamin A to gains in weight are given for males and females separately for the 3-week feeding period.
4. Statistical analysis of the data obtained from the experiments to determine the curve of response indicated (1) that seven or eight male rats are required to demonstrate a difference of 20 percent in gain of weight or of vitamin A values, (2) that although the use of seven or eight female rats may be used to demonstrate a difference of 20 percent gain in weight, only a difference in vitamin A values of 50 percent may be determined with this number, because of the small growth increments. For females the weight gains must be kept at about those obtained when 2.0 I.U. of vitamin A or less is fed. For the males the curve of reference may be used up to 3 I.U.
5. Statistical analyses of the pooled data for all rats used, over a period of approximately 3 years, as positive controls, and fed sufficient reference cod liver oil to supply 1.5 I.U. of vitamin A daily indicated that because of greater variability in weight gains due to many uncontrollable

factors, the number of rats necessary for a significant vitamin A assay (20 percent of the true value) would be greater. For males about 12 rats would be required. The same number of female rats would show a difference of only 50 percent of the vitamin A value, although this number would demonstrate a 20 percent difference in gain in weight.

TABLE A. Summary of the results of feeding rats a vitamin A-free diet and graded doses of Reference Cod Liver Oil as a source of vitamin A for 3 weeks and 5 weeks (13 males and 13 females in each group of rats).

DAILY SUPPLEMENT VITAMIN A	AVERAGE WEIGHTS				MEAN GAINS WITH STANDARD ERRORS			
	At weaning		At depletion		In 3 weeks		In 5 weeks	
	M	F	M	F	M	F	M	F
	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I.U.								
0.0	39	36	103	91				
0.5	39	37	100	91	16±2.3	22±2.1	21±2.7	24±2.8
1.0	39	37	102	87	33±2.5	37±1.6	52±3.8	53±3.1
2.0	38	36	96	91	64±2.9	51±1.6	107±3.7	74±2.2
3.0	38	35	103	84	68±2.5	54±2.8	109±6.0	78±4.3
4.0	37	35	99	84	80±3.0	54±2.0	127±4.9	79±2.6
8.0	38	35	98	90	91±2.7	58±2.1	143±4.6	85±4.7

TABLE B. The standard errors and "t" values of differences between the weight gains of groups of rats fed supplements of Reference Cod Liver Oil.

INTERVALS OF SUPPLEMENTS	3 WEEKS		5 WEEKS	
	Difference	"t" Value	Difference	"t" Value
	Gm.		Gm.	
I.U.				
Males				
0.5-1.0	17±3.40	5.0	31±4.66	6.7
1-2	31±3.83	8.1	55±5.30	10.4
2-3	4±3.83	1.0	2±7.05	—
3-4	12±3.91	3.1	18±7.75	2.3
4-8	11±4.04	2.7	16±6.72	2.4
Females				
0.5-1.0	15±2.64	5.7	29±4.18	6.9
1-2	14±2.26	6.2	21±3.81	5.5
2-3	3±3.22	—	4±4.83	—
3-4	0±3.44	—	1±5.03	—
4-8	4±2.91	1.4	6±5.37	1.1

TABLE C. Analysis of variance of gain in weight of thirteen rats in each of six vitamin A treatments after a 3-week feeding period.

SOURCE OF VARIANCE	DEGREES OF FREEDOM	VARIANCE
Males		
Total	77	
Between levels	5	10.639
Error	72	92.5
Standard error, gain in weight of a single rat		9.62
Females		
Total	77	
Between levels	5	2.499
Error	72	55.7
Standard error, gain in weight of a single rat		7.46

TABLE D. Analysis of variance of gain in weight of rats fed Reference Cod Liver Oil* (1.5 I.U. vitamin A daily).

SOURCE OF VARIANCE	DEGREES OF FREEDOM	VARIANCE
<i>Males</i>		
Total	67	
Between experiments	9	440
Error	58	109
Standard error, gain in weight of a single rat		10.44
<i>Females</i>		
Total	101	
Between experiments	11	64.4
Error	90	65.2
Standard error, gain in weight of a single rat		8.07

* The data were obtained during a period of 3 years. Each rat used as a positive control was given the cod liver oil supplement for the 3-week feeding period.

VITAMIN A ASSAYS USING VITAMIN A ACETATE AND TOCOPHEROL

When rats of the same colony on which the curve of response had been established showed increasingly poor gains in weight when fed the reference cod liver oil, a change was made to vitamin A acetate as a standard and some other details of the procedure were changed. Briefly the method used was as follows:

At 21 days of age young animals were put on a vitamin A-free diet, consisting of vitamin A-free casein, 18 percent; dried yeast, 10 percent; salt mixture, 2.5 percent; olive oil, 5 percent; and cornstarch, 64 percent. Thiamine (10 micrograms per gram of diet) and irradiated ergosterol (5 I.U. per gram of diet) were added.

At depletion the animals were fed the supplements for a period of 3 weeks as previously. Vitamin A for the positive controls was furnished by an olive oil solution of vitamin A acetate.⁵ In addition, they received a 0.1 milligram supplement of tocopherol⁶ in olive oil, in accordance with the recommendation of Hickman *et al.* (13). The rats receiving food supplements (containing carotene) were likewise given tocopherol, amounting to 0.5 milligram daily, as recommended by Harris *et al.* (9).

The curve of response previously established was not used as a reference for the procedure just outlined, but a direct comparison of weight gains of the control groups and the test rats was made.

⁵ Crystalline vitamin A acetate was purchased from Distillation Products, Inc., Rochester, New York.

⁶ A concentrate of mixed tocopherols donated by Distillation Products, Inc., Rochester, New York.

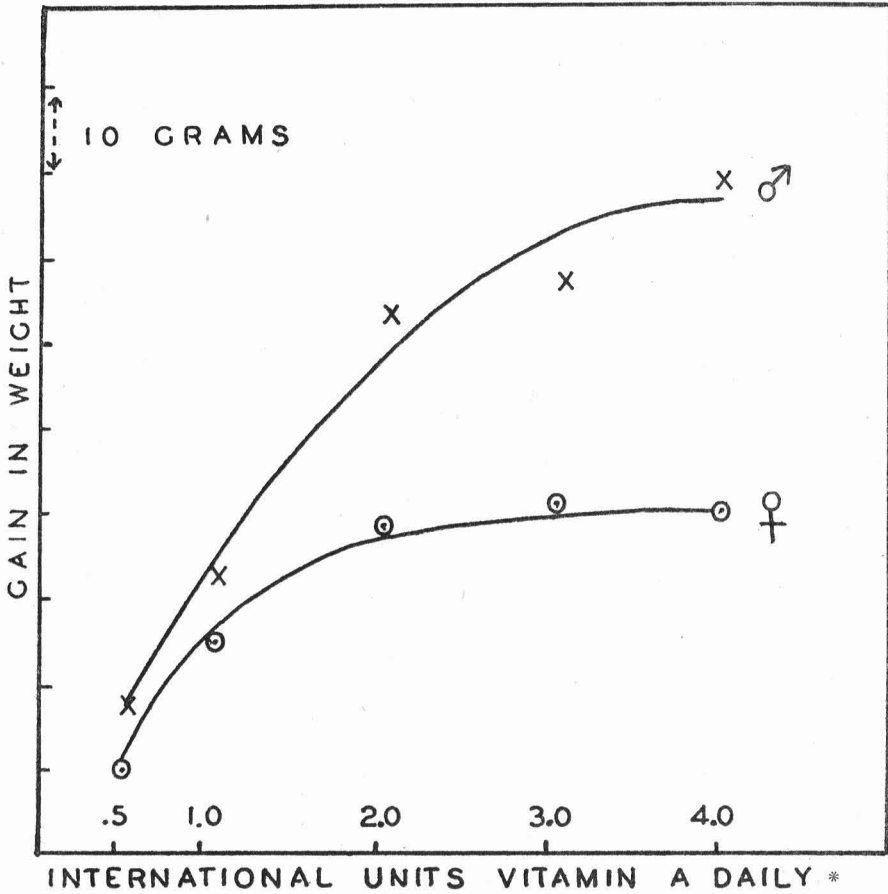


FIGURE 1. Calculated curves of response for male and female rats relating gains in weight of rats with doses of vitamin A of Reference Cod Liver Oil Number II.

* It should be noted that the dosage of vitamin A for the curve of response is given in terms of daily supplements to correspond with tables A and B (p. 41) but that for the data in table E (p. 49) these have been changed to weekly supplements. That is, 1.0 I.U. daily corresponds to 6.0 I.U. weekly, and 1.5 I.U. daily corresponds to 9 I.U. weekly.

APPENDIX II

DESCRIPTION AND PREPARATION OF FOODS USED FOR ASCORBIC ACID ASSAYS GIVEN IN TABLES 1, 3, 4, AND 9

If no description is given here it may be assumed (1) that the food was grown or purchased on Oahu, (2) that the sample was of good market quality, (3) that the variety was unknown unless given in the tables, and (4) that the portions taken for duplicate assays constituted representative samples of the whole. Methods and periods of cooking for all foods are given on pages 10 and 11.

The word "average" used in the descriptions which follow refers to the figures for ascorbic acid which appear in table 1.

Asparagus, green—Mary Washington variety. Average of two samples obtained 4 years apart.

Avocado—Six samples of West Indian Race (thin skin, summer and fall ripening), one sample Guatemalan Race (Haley variety). Each sample represented an individual fruit from a different source.

Banana—Bio-assay, six guinea pigs for Bluefields, six for Chinese bananas, and six for positive controls fed three levels of pure ascorbic acid; fruit from Poamoho Farm. Six chemical assays: Bluefields from Kaneohe; average of eight fruit from same hand. Brazilian from Poamoho; average of eight fruit from same hand. Chinese from Poamoho; average of eight fruit from same hand. Icecream from Kaneohe; average of seven fruit from same hand.

Banana, cooking (Popoula Kaio)—Raw, two bananas from two hands. Cooked in boiling water 15 to 20 minutes, two bananas from two hands.

Banana bud—Chinese banana. Average of two buds, mixture of flowers from inner and outer portions plus the tender bracts. Flowers and bracts were soaked 5 minutes in a 4 percent solution of C.P. NaCl, washed in tap water, rinsed twice in distilled water, drained, wiped dry, and assayed. The cooked sample was steamed for 5 minutes.

Bean sprouts, mung—Average of three raw samples and of two samples cooked 5 minutes. One sample was assayed after 22, 46, and 70 hours storage in refrigerator and at room temperature.

Beans, green—Lualualei, average of three samples from different areas, raw and cooked.

Beans, lima—Average of two varieties (Florida Speckled and Fordhook Climbing) from different sources, raw and cooked.

Belembe (Tahitian taro)—Average of two samples from the same source assayed 4 years apart.

Bittermelon fruit—Cross sections were taken from three fruits after discarding seeds and inner membrane. Sections of the same fruits were cooked.

Bittermelon leafy tips—One sample tested 1 hour after harvesting.

Blackberries—Introduced evergreen variety, growing wild in Volcano District, Hawaii. Average of two samples gathered 1 month apart. The berries were sent to Honolulu by plane and were assayed 24 and 36 hours after picking.

Breadfruit—Mature green, raw—average of two samples, same tree, different years. Ripe, raw—average of five samples from the same tree. Ripe, cooked—baked whole for one hour at 350° F.

Cabbage, head—Average of two samples, varieties unknown, assayed same day or the day after harvest, raw and cooked.

Cabbage, Chinese (celery cabbage)—One market sample, probably several days after harvest, raw and cooked. The value, which was approximately half that of the fresh sample, is not included in table 1. One garden fresh sample, raw and cooked.

Cactus fruit—One sample, deep red color. Seeds and flesh assayed separately from rind, practically the same value for both.

Carambola—Average of three separate assays, each on three or four fruits. Each lot of fruit from a different source, different years. One lot was sweet, one semi-sweet, and one sour.

Carissa—One assay on five fruits from Wilhelmina Rise.

Carrots—Raw, average of five samples of Imperator and five of Danvers Half Long; wedges from each of eight carrots for each assay. Cooked, average of two samples of each variety, using wedges from the same carrots as used for raw.

Celery—One sample Cornell No. 6. Inner and outer stalks without leaves, raw and cooked, from one bunch.

Chard—One sample from market, blade and petioles used.

Chayote—One fruit from Station plot, raw, unpeeled.

Coconut—Immature, "spoon stage" of white meat, one sample.

Coconut water—The water from within 11 coconuts was used. The nuts varied in maturity from one with almost mature white meat, through the various soft stages, to no meat.

Cowpeas, fresh pods—Average of three samples, two green, one red and green, fresh and cooked.

Cowpeas, tender tips—From an all-green variety of cowpea; 4 to 6 inches of tender tips were used.

Cucumber—Pared, average of two samples, each a single cucumber. Unpared, one cucumber.

Daikon—Average of two assays. One assay, wedge-shaped sections from three roots; another from two roots.

Eggplant—Round variety, average of two individual fruits, one pared, one unpared, each raw and cooked. Long variety, average of four individual fruits, two pared, two unpared, raw and cooked.

Figs—Average of two samples, 2 different years. Each sample consisted of four figs.

Grapes, Isabella—Samples taken from several clusters of one lot of grapes for whole and seeded pulp.

Guava, Cattley—Red (Strawberry), average of two lots of fruit from two sources; 10 fruits in one lot; 11 fruits in the other. Pale yellow, one sample composed of six fruits from Mountain View, Hawaii.

Horseradish tree, leaflets and tender tips—One sample; the leaflets were stripped from stems and added to tips.

Horseradish tree pods—One sample, made up of sections taken from stem ends, center, and tip ends of eight pods. All hard outer covering and fibers were removed from pods $\frac{1}{4}$ to $\frac{1}{2}$ inch in diameter.

Java plum (jambolan)—Purple-fleshed fruit from Manoa Valley, one sample of 10 small and 10 large fruits. White-fleshed fruit from Molokai, one sample of 10 small and 10 large fruits.

Ketambilla (Ceylon gooseberry)—Average of two samples from two sources harvested 1 month apart.

Lettuce, Manoa (semi-head)—Average of two samples from two sources. Four to six leaves in each duplicate.

Limes—Juice from four to eight limes was used for each variety. The juice was strained through one layer of cheesecloth.

Litchi, Kwai Mi—One sample of 12 fruits.

Lotus root—Raw, average of two samples; each sample made up of cross sections from two lobes, each lobe from a different root. Cooked, one sample from two roots.

Mango—Sections from two to six mangoes were used for each duplicate sample. For the Pirie variety, mangoes were obtained from three sources, at three different seasons. Two to four fruits were used for each duplicate.

Milk, raw—Average of six samples from University Dairy on 6 different days during January, 1945.

Milk, pasteurized—Average of seven samples; five from the University Dairy on 5 different days in February, 1945, two samples from commercial dairies during the same month.

Mountain apple—Average of two samples from two seasons, four apples in each sample.

Ohelo berry—Average of two samples from the Volcano District, Hawaii, two seasons.

Okra—Both raw and cooked samples taken from one lot of five pods, 4 to $6\frac{1}{2}$ inches long.

Onions, dry—Average of one sample each from two varieties, White Babosa and Yellow Bermuda. Each sample was taken from three onions.

Orange juice, Hawaiian—Average of 13 oranges from three lots of fruits from two sources.

Papaya—Averages of 45 samples from Poamoho (Experiment Station Farm), and 40 samples from Kailua, collected at weekly intervals over a period of 1 year, except for the month of August.

Passion fruit, yellow variety—Average of two samples from two sources, two seasons. Each sample taken from four fruits. Data on pulp with seeds and strained juice were similar.

Pepper, green bell—Average of two samples, two seasons, from two sources, raw and cooked. One sample taken from two peppers, one from three peppers.

Pineapple, fresh (Cayenne variety)—Average of nine samples; each sample taken from one fruit.

Plum, red (Methley variety)—One sample taken from five plums, grown in Volcano District, Hawaii. Sent to Honolulu by plane. Assayed 30 hours after picking.

Poha—Average of two samples from Volcano District, Hawaii. Assayed 36 hours after picking. Each sample made up of eight or nine representative berries.

Pomelo—One sample taken from three fruit.

Potato, Bliss Triumph—Average of two samples (raw), two seasons, 3 years apart, assayed at time of harvesting and after 2 months' storage. One sample cooked in skins, in boiling salted water, before and after storage. Each sample taken from four potatoes. One lot of stored potatoes assayed at bi-weekly intervals for 12 weeks.

Potato, California (Shafter)—One sample taken from three potatoes.

Roselle—One sample, composed of the fleshy, red calyx, from a number of fruits.

Sesbania flowers, white—One sample. Buds and opened flowers were used. Stem, calyx, and pistil were discarded. Cooked by immersion in boiling water for 1 minute.

Soursop—One sample of juice prepared by squeezing pulp in two thicknesses of cheesecloth.

Soybeans—One sample raw and cooked. Variety unknown. Values for beans cooked in and out of the pods were similar.

Squash, summer—Pattypan—average of two samples, each sample taken from six squash. *Zucchini*—three samples, each taken from three or four squash. Cooked and raw samples were taken from the same fruit.

Squash (winter-type) and pumpkin—Flowers—one sample. Three flowers used for each duplicate. Cooked by dipping in boiling water for 1 minute. *Fruit*—Average of two samples, Filipino and Kona Crepe varieties, raw and cooked. Sample of Filipino variety from one fruit, Kona Crepe from two fruits. *Leafy tender tips*—one sample, raw and cooked. Used 2 to 3 inches of tips and other tender leaves.

Strawberry—Red (cultivated)—one sample (a mixture of small and large berries) was assayed 24 hours after picking. *White (small, wild)*—from Kilauea National Park, Hawaii. One sample, assayed 24 hours after picking.

Surinam cherry—Average of three samples from three sources.

Sweetpotato—Tantalus variety. One sample taken from three potatoes raw and cooked. Steamed after paring.

Tangelo—One sample of juice prepared from three fruits.

Taro, Hawaiian, corm—One sample, two corms raw, two corms cooked by steaming 45 minutes at 15 pounds pressure.

Taro, Hawaiian, leaves (luau)—Two samples, stems and heavy midrib removed, steamed 1 hour.

Taro, Japanese (Dasheen)—One sample made up of five corms. Second sample made up of three corms.

Tamarind—Two samples of green tamarind; one had no ascorbic acid, a second contained 2 milligrams per 100 grams. One sample of ripe tamarind.

Tomato, pear-shaped—Average of three samples, three sources and three seasons. Each sample was taken from four fruits, one was peeled and two were unpeeled.

Tomato, Globe—Average for and range of values for 72 samples of good market grade fruit. Equatorial sections from three to six tomatoes constituted each sample. All peeled.

Tomato, Globe (imported)—Average of 12 samples over a period of 4 months. Each sample taken from two to four tomatoes, most samples from three tomatoes, all peeled.

Tree fern fronds—Young, uncurled fronds of a large and a small variety were obtained from Kilauea National Park, Hawaii. Brought to Honolulu by plane and refrigerated until assayed, raw and cooked, 36 hours after picking. Brown floss and fibrous material removed from large fronds.

Vi—One sample taken from two small and two medium fruits.

Watercress—Average of two samples, tips and tender leaves, from two sources, two seasons, raw and cooked.

Watermelon—Average of 14 separate assays on flesh from stem ends, center, and bud ends of two melons, one from Maui and one from Oahu.

Yam bean root—Sample taken from three roots.

APPENDIX III

TABLE E. Food supplements, weights, and mean gains of rats used for vitamin A bio-assays.

WEEKLY SUPPLEMENT	RATS, NUMBER AND SEX	AVERAGE WEIGHTS			MEAN GAINS IN 3 WEEKS Gm.
		At weaning Gm.	At depletion Gm.	Final	
Negative control	2 M	} 38	72	4 died	---
Negative control	5 F				
Ref. C.L.O., 9 I.U.*	6 M	38	75	108	33
Ref. C.L.O., 6 I.U.	5 F	38	69	96	27
Tomato, pear-shaped (raw), 0.5 gram	4 M	38	70	93	23
Beans, green (Lualualei), 0.7 gram	4 F	37	72	112	40
Watercress (raw), 0.2 gram	6 F	39	76	113	37
Avocado (winter-type), 1.5 grams	4 M	38	74	96	22
Negative control	2 M	} 37	65	4 died	---
Negative control	5 F				
Ref. C.L.O., 6 I.U.	6 F	38	68	91	23
Paiai (cooked taro corm), 9 grams	6 F	38	64	86	22
Negative control	1 M	} 35	66	1 died	---
Negative control	3 F				
Ref. C.L.O., 9 I.U.	7 F	36	64	106	42
Potatoes, Bliss Triumph, 18 grams	7 F	35	64	83	19
Beans, green lima, 1.0 gram	6 F	34	68	98	30
Negative control	5 M	} 39	80	2 died	---
Negative control	3 F				
Ref. C.L.O., 9 I.U.	4 M	38	75	118	43
Ref. C.L.O., 9 I.U.	8 F	38	72	115	43
Asparagus, 0.6 gram	6 F	40	63	118	55
Sweetpotato (light yellow), 0.3 gram	6 F	37	68	107	39
Negative control	1 M	} 39	80	71	---
Negative control	4 F				
Ref. C.L.O., 9 I.U.	7 F	37	79	122	43
Cabbage (head), 12 grams	6 F	36	76	123	47
Cabbage, Chinese, 12 grams	5 F	37	77	110	33
Eggplant (round), 12 grams	6 F	36	77	134	57
Kiawe bean meal, 6 grams	6 F	38	85	109	24
Negative control	4 M	42	95	76	---
Ref. C.L.O., 9 I.U.	6 M	37	81	123	42
Ref. C.L.O., 9 I.U.	6 F	40	82	121	39
Watercress (raw), 0.3 gram	6 M	38	80	133	53
Watercress (raw), 0.2 gram	6 F	40	82	129	47
Negative control	5 F	38	82	2 died	---
Ref. C.L.O., 9 I.U.	5 M	38	82	121	39
Ref. C.L.O., 9 I.U.	7 F	34	72	112	40
Pineapple bran, 0.45 gram	6 M	40	86	159	73
Pineapple bran, 0.3 gram	7 F	35	73	124	51
Taro leaves (luau), 0.1 gram	7 F	37	75	128	53
Negative control	6 F	38	90	89	---
Ref. C.L.O., 9 I.U.	9 F	38	85	128	43
Spinach, Chinese (amaranth), 0.15 gram	6 F	39	85	139	54
Squash, deep yellow, 0.06 gram	6 F	37	84	123	39
Sweetpotato, deep yellow, 0.1 gram	7 F	37	87	136	49

* International Units of United States Pharmacopeia Reference Cod Liver Oil.

TABLE E (continued)

WEEKLY SUPPLEMENT	RATS, NUMBER AND SEX	AVERAGE WEIGHTS			MEAN GAINS IN 3 WEEKS
		At weaning	At depletion	Final	
Negative control	2 M	Gm.	Gm.	Gm.	Gm.
Negative control	6 F	39	94	81	---
Ref. C.L.O., 9 I.U.	9 M	40	93	139	46
Ref. C.L.O., 9 I.U.	9 F	36	80	122	42
Bean sprouts, mung, 12 grams	9 M	40	96	165	69
Cabbage, white mustard, 0.2 gram	9 F	37	80	133	53
Cabbage, green mustard, 0.2 gram	9 F	38	84	141	57
Turnip greens, 0.15 gram	8 F	37	79	131	52
Cowpeas, shelled, 0.8 gram	4 F	36	76	112	36
Negative control	9 F	37	90	83	---
Ref. C.L.O., 9 I.U.	5 M	33	86	134	48
Ref. C.L.O., 9 I.U.	7 F	35	85	132	47
Onions, green, 0.25 gram	6 M	39	91	167	76
Carrots, 0.15 gram	7 F	33	81	147	66
Lettuce, Manoa (raw), 0.1 gram	7 F	33	81	122	41
Beet greens, 0.15 gram	8 F	35	83	135	52
Negative control	2 M	}	94	3 died	---
Negative control	10 F				
Ref. C.L.O., 9 I.U.	7 M		97	159	62
Broccoli, 0.2 gram	8 M		94	175	81
Negative control	5 F	34	93	92	---
Ref. C.L.O., 9 I.U.	10 F	35	84	129	45
Beans, yellow wax, 4 grams	7 F	37	90	138	48
Chard, 0.13 gram	8 F	37	87	131	44
Negative control	5 M	}	92	2 died	---
Negative control	5 F				
Ref. C.L.O., 9 I.U.	7 M		84	134	50
Ref. C.L.O., 9 I.U.	10 F		76	114	38
Taro, Japanese, 12 grams	11 F	36	75	114	39
Lotus root, 18 grams	6 M	38	88	97	9
Guava pulp, fresh, 6 grams	7 M	36	83	151	68
Guava pulp, cooked, 9 grams	9 M	36	86	181	95
Soybeans, green, 1.5 grams	9 F	34	73	127	54
Belembe, 0.1 gram	9 F	35	76	127	51
Negative control	8 M	}	91	4 died	---
Negative control	3 F				
Ref. C.L.O., 9 I.U.	10 M		80	129	49
Ref. C.L.O., 9 I.U.	11 F		77	123	46
Beans, green, Kentucky Wonder, 0.9 gram	8 M	35	85	166	81
Beets, 12 grams	7 M	34	79	113	34
Breadfruit, 18 grams	9 M	34	82	155	73
Egg yolk, 0.3 gram	8 F	33	73	126	53
Green pepper, 0.9 gram	7 F	33	69	119	50
Milk, fresh whole, 4.5 milliliters	8 F	34	75	127	52
Negative control	7 M	}	102	10 died	---
Negative control	6 F				
Ref. C.L.O., 9 I.U.	8 M		99	155	56
Ref. C.L.O., 9 I.U.	11 F		91	134	43
Banana, baking, 2 grams	9 M	37	92	178	86
Carambola, 2 grams	7 M	38	102	193	91
Corn, 18 grams	9 F	37	87	148	61
Chayote, 3 grams	9 M	38	99	169	70
Pineapple, 12 grams	10 F	37	86	136	50
Avocado, summer, 3 grams	9 M	38	103	139	36
Okra, 2 grams	9 F	37	93	156	63

TABLE E (continued)

WEEKLY SUPPLEMENT	RATS, NUMBER AND SEX	AVERAGE WEIGHTS			MEAN GAINS IN 3 WEEKS
		At weaning	At depletion	Final	
		Gm.	Gm.	Gm.	Gm.
Negative control	9 M	} 38	107	9 died	---
Negative control	5 F				
Ref. C.L.O., 12 I.U.*	5 M	38	104	143	39
Ref. C.L.O., 9 I.U.	6 F	35	97	126	29
Avocado, winter, 4 grams	7 M	36	99	151	51
Papaya, 0.6 gram	9 F	38	98	147	49
Poha, 0.4 gram	5 M	37	102	155	53
Banana, Bluefield, 2 grams	8 F	35	94	131	36
Negative control	2 M	} 36	87	3 died	---
Negative control	4 F				
Ref. C.L.O., 12 I.U.	9 M	35	90	131	41
Ref. C.L.O., 9 I.U.	8 F	33	78	103	25
Orange juice, Kona, 18 milliliters	7 F	34	73	105	32
Passion fruit juice, 6 milliliters	7 M	36	92	168	76
Negative control	9 M	36	67	5 died	---
Ref. C.L.O., 18 I.U.	9 M	35	67	149	82
Carissa, 6 grams	7 M	34	60	58	-2
Negative control	3 M	} 35	98	3 died	---
Negative control	3 F				
Vitamin A acetate, 12 I.U.	6 F	37	96	133	37
Vitamin A acetate, 18 I.U.	8 F	35	101	144	43
Mango, 0.2 gram	11 F	37	99	136	37
Mango, 0.4 gram	13 F	36	102	154	52
Negative control	3 F	} 39	125	109	---
Negative control	1 M				
Vitamin A acetate, 18 I.U.	5 F	37	114	171	57
Surinam cherry, 0.7 gram	7 F	38	113	167	55
Negative control	3 F	} 35	97	2 died	---
Negative control	2 M				
Vitamin A acetate, 18 I.U.	4 M	37	105	143	48
Vitamin A acetate, 24 I.U.	7 M	36	103	165	62
Zucchini, 3 grams	11 M	37	105	160	55
Zucchini, 4 grams	12 M	37	106	175	69
Negative control	5 M	} 37	115	4 died	---
Negative control	3 F				
Vitamin A acetate, 18 I.U.	9 M	36	107	167	60
Vitamin A acetate, 24 I.U.	8 M	37	116	191	71
Horseradish tree pods, 3 grams	9 M	35	101	166	65
Horseradish tree pods, 4 grams	9 M	37	120	196	76
Horseradish tree pods, 6 grams	10 M	35	110	195	85
Negative control	2 M	} 35	101	2 died	---
Negative control	4 F				
Vitamin A acetate, 18 I.U.	3 M	39	113	158	44
Vitamin A acetate, 18 I.U.	4 F	36	99	138	39
Vitamin A acetate, 24 I.U.	6 M	37	105	157	52
Plums, 3 grams	3 M	38	116	156	35
Plums, 3 grams	6 F	34	88	125	37
Plums, 4 grams	3 M	38	106	154	48
Plums, 4 grams	6 F	35	97	129	32

* Potency of this and following lots of Ref. C.L.O. seemed low.

APPENDIX IV

TABLE F. Food supplements, weights, and mean gains of rats used for thiamine bio-assays.

DAILY SUPPLEMENT	NUMBER OF RATS	AVERAGE WEIGHTS			MEAN GAINS IN 3 WEEKS
		At Weaning	At Depletion	Final	
		Gm.	Gm.	Gm.	
First Depletion					
Negative control	7	43	69	54	---
Thiamine, 3 micrograms	13	45	73	98	25
Bean sprouts, mung, 3 grams	13	44	74	99	25
Watercress, 4 grams	13	45	72	97	25
Negative control	6	41	62	45	---
Thiamine, 3 micrograms	12	43	67	89	22
Avocado (winter type), 4 grams	13	42	66	123	57
Beans, green (Lualualei), 4 grams	12	41	66	104	38
Tomato, pear-shaped, raw, 3 grams	12	42	66	97	31
Negative control	9	42	66	45	---
Thiamine, 3 micrograms	10	41	67	88	21
Taro leaves (luau), 2 grams	13	43	72	92	20
Paiai (cooked Hawaiian taro corm), 4 grams	13	45	72	98	26
Negative control	17	42	72	50	---
Thiamine, 3 micrograms	15	42	73	97	24
Cowpeas (Blackeye), shelled, 1.5 grams	13	43	76	119	43
Sweetpotato (v. Tantalus), 3 grams	13	43	76	103	27
Negative control	10	40	83	62	---
Thiamine, 3 micrograms	17	40	78	111	33
Banana, cooking, 5 grams	13	40	82	104	22
Milk, raw, 8 milliliters	13	43	79	104	25
Peanut, raw, 0.5 gram	13	39	77	109	32
Peanut, roasted in oil, 1 gram	13	39	78	95	17
Negative control	8	35	61	40	---
Thiamine, 3 micrograms	15	39	66	95	29
Taro, Japanese (Dasheen)	13	32	61	104	43
Negative control	11	38	64	48	---
Thiamine, 3 micrograms	15	38	66	107	41
Onions, green, 5 grams	12	38	67	107	40
Carrots, 5 grams	12	38	69	102	33
Cabbage (head), 5 grams	12	37	67	92	25
Negative control	12	38	71	48	---
Thiamine, 3 micrograms	16	38	69	107	38
Chard, 5 grams	12	38	66	103	37
Beet greens, 5 grams	12	38	68	100	32
Negative control	7	40	69	52	---
Thiamine, 3 micrograms	13	41	72	100	28
Cowpeas, entire pod, 2 grams	12	40	68	96	28
Squash (winter-type), 1.5 grams	11	40	70	88	18
Yeast, dried (H.S.P.A. Expt. Sta.), 0.05 gram	13	41	71	95	24
Negative control	17	42	76	54	---
Thiamine, 3 micrograms	18	42	76	112	36
Mango, Pirie, 3 grams	13	40	75	98	23
Negative control	10	43	81	52	---
Thiamine, 3 micrograms	15	43	78	100	22
Avocado (winter-type), 3 grams	14	42	77	110	33
Banana, Bluefield, 6 grams	14	43	77	78	< 1
Orange juice, Kona, 4 milliliters	12	43	76	89	12
Papaya, 6 grams	12	42	77	90	12

TABLE F (continued)

DAILY SUPPLEMENT	NUMBER OF RATS	AVERAGE WEIGHTS			MEAN GAINS IN 3 WEEKS
		At Weaning	At Depletion	Final	
		Gm.	Gm.	Gm.	
Negative control.....	8	41	74	(6 dead)	----
Thiamine, 3 micrograms.....	12	41	70	93	23
Zucchini, 5 grams.....	11	42	76	96	20
Second Depletion					
Negative control.....	2	91	91	62	----
Thiamine, 3 micrograms.....	11	96	91	104	13
Winter avocado, 3 grams.....	12	95	92	116	24
Beans, green (Kentucky Wonder), 3 grams.....	12	97	94	107	13
Negative control.....	5	105	99	71	----
Thiamine, 3 micrograms.....	12	95	91	105	14
Potatoes, Bliss Triumph, 3 grams.....	13	93	90	116	26
Beans, green lima, 1 gram.....	12	92	89	94	5
Beans, yellow wax, 4 grams.....	12	88	85	99	14
Negative control.....	12	44	77	63	----
Thiamine, 3 micrograms.....	16	43	72	106	34
Spinach, Chinese (amaranth), 4 grams.....	15	43	74	87	13
Thiamine, 4 micrograms.....	15	100	95	143	48
Asparagus, 3 grams.....	14	100	96	147	51
Broccoli, 4 grams.....	13	101	96	138	42
Cabbage, white mustard, 5 grams.....	13	102	99	114	15
Cabbage, green mustard 5 grams.....	13	101	98	131	33
Thiamine, 4 micrograms.....	15	88	85	124	39
Breadfruit, 4 grams.....	13	91	87	132	45
Beets, 6 grams.....	12	87	84	92	8
Thiamine, 4 micrograms.....	13	95	94	132	38
Belembe, 3 grams.....	12	93	91	112	21
Eggplant, round, 4 grams.....	13	92	90	124	34
Soybeans, green (raw), 1.5 grams.....	13	93	91	135	44
Soybeans, green (cooked), 2 grams.....	13	92	89	125	36
Thiamine, 4 micrograms.....	20	105	102	148	46
Avocado (summer-type), 3 grams.....	13	104	102	128	26
Coconut (fresh mature), 3 grams.....	13	106	106	145	39
Carambola, 5 grams.....	13	106	104	119	15
Okra, 3 grams.....	13	102	100	132	32
Chayote, 7 grams.....	6	111	106	110	4
Coconut water, 10 milliliters.....	5	106	104	113	9
Thiamine, 4 micrograms.....	16	103	101	145	44
Daikon, 5 grams.....	15	103	101	118	17
Lotus root, 3 grams.....	13	99	98	129	31
Negative control.....	9	92	90	77	-16
Thiamine, 4 micrograms.....	13	86	81	119	38
"Black rice" (cooked), 3 grams.....	14	94	90	120	28

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